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VOL. 17, SEC. C. JUNE, 1939 NUMBER 6

MICROBIC DISSOCIATION OF LACTIC ACID STREPTOCOCCI[1]

BY OLGA OKULITCH[2]

Abstract

The induction of microbic dissociation of the lactic acid streptococci by the presence in the medium of a carbohydrate with a specific configuration has been studied.

Strains of *Streptococcus lactis* and *Streptococcus cremoris* have been shown to undergo dissociative changes when grown in the presence of glucose. Lithium chloride and phenol have been found to be without influence. The change from the *S*- to *R*-form was accompanied by loss of lactose-fermenting ability.

The cultural, colonial, and biochemical characters of the organisms at different stages of variation have been described.

It is suggested that the organism must be in a susceptible condition before dissociation can be induced.

In a preliminary note (11), the inhibitory power of specific carbohydrates on the ability of lactic acid streptococci to form acid from lactose in milk or in broth was described. It was suggested that this restraining influence on acid formation was related to the stereoisomeric structure of the hexoses. Although no reference was made at the time to the influence of the sugars on the morphological and cultural appearance of the organism, distinct changes in these characters, which seemed to be connected in some way with the loss of lactose-fermenting ability, were observed. A review of the literature on the question of bacterial dissociation suggested that the cultural and colonial variations which we had encountered were of the same nature as those described by Hadley (5, 6). As the work progressed, it appeared probable that we were dealing with a phenomenon of dissociation in the lactic acid streptococci and that the microbic dissociation had been induced by the presence in the medium of a carbohydrate having a specific configuration.

The work reported upon herein is a detailed study of the bacterial dissociation of strains of lactic acid streptococci grown in media containing the carbohydrate, glucose. In an attempt to establish the sequence of variation in cultural and colonial characters, several strains of *Strep. cremoris* and *Strep. lactis* have been employed. In addition to the *Strep. cremoris* strain No. 142, described in a previous paper (11), the following organisms were selected for intensive study: *Strep. cremoris* 114, *Strep. lactis* 146, and *Strep. lactis* 232 (10). Certain observations on microbic dissociation have also been carried out in the case of several other streptococcus cultures.

[1] *Manuscript received March 8, 1939.*
Contribution from the Department of Dairying, The University of British Columbia, Vancouver, Canada.

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Experimental

The induction of dissociation and the accompanying inhibition of lactose-fermenting ability were brought about by serial cultivation of a vigorous culture of the strain under investigation in casein digest broth (4) and in peptonized milk broth (4), the broth in each case containing 2% glucose. Serial transfers in each broth were made at 48-hr. intervals. At every transfer, milk also was inoculated with the culture from the sugar broths, and the time required to clot the respective milk tubes determined. At frequent intervals poured plates on Difco peptonized milk gelatin were made from the broth cultures. The colonies were examined with a low power microscope, giving a ×40 magnification.

Cultural Characters

Discussion

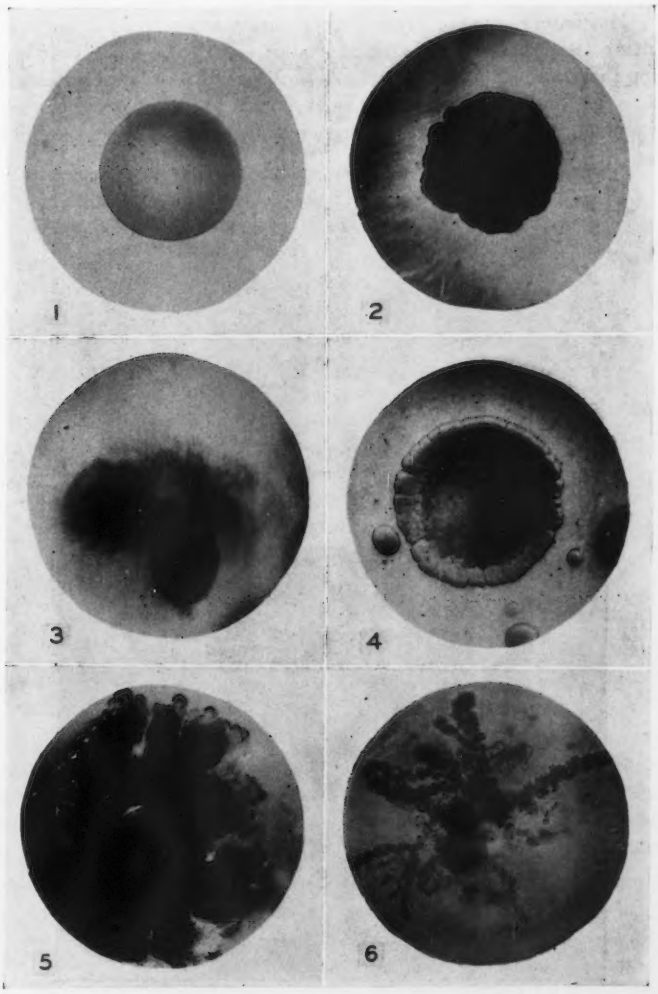
Dissociation among lactic acid streptococci results in the development of a variety of colonial types. The commonest initial change is from a round, smooth colony (Fig. 1) to a slightly lobate form (Fig. 2). If these lobate colonies are allowed to age for one to two weeks, outbursts consisting of a very fine rough growth from one or more points in the margin of the colony are formed (Fig. 3). In certain instances, aging of the lobate colonies leads to the formation of a fringe of daughter colonies, or papillae, around the entire colony (Fig. 4). At the time that these changes in the colony form appear it is possible that the organism is in the transition stage from the *S*- to the *R*-type. The picking of these intermediate-type colonies never gives a broth-culture characteristic of a pure smooth or rough strain. On plating this culture, a mixture of smooth, lobate, and pure rough colonies is obtained. A pure rough culture of a lactic acid streptococcus plated on peptonized milk gelatin usually gives rise to a rough, filamentous, spider-like colony (Fig. 6). With certain strains, however, a more compact, although definitely filamentous type of colony, is to be seen (Fig. 5).

On several occasions, in addition to the characteristic *S*- and/or *R*-types of colonies which appeared on peptonized milk gelatin plates, very small pin-point colonies were also to be seen. On inoculation of these colonies into broth, a cloudy growth resulted, which, on transfer, failed to produce acid in milk. On replating, the culture reverted to the normal *S*-form, and evidenced the cultural characters of the original strain, including the ability to form acid from lactose in milk. Colonies of this type have been described by Dutton (3) and also by Staryghina (13).

The morphological appearance of the pure *S*-form of lactic acid streptococci is characterized by a regularity in the size and shape of the cells, which occur usually in pairs or short chains (Fig. 7). On dissociating to the stable *R*-form, the culture becomes decidedly long-chained, the cells at times being very irregular in shape (Fig. 8).

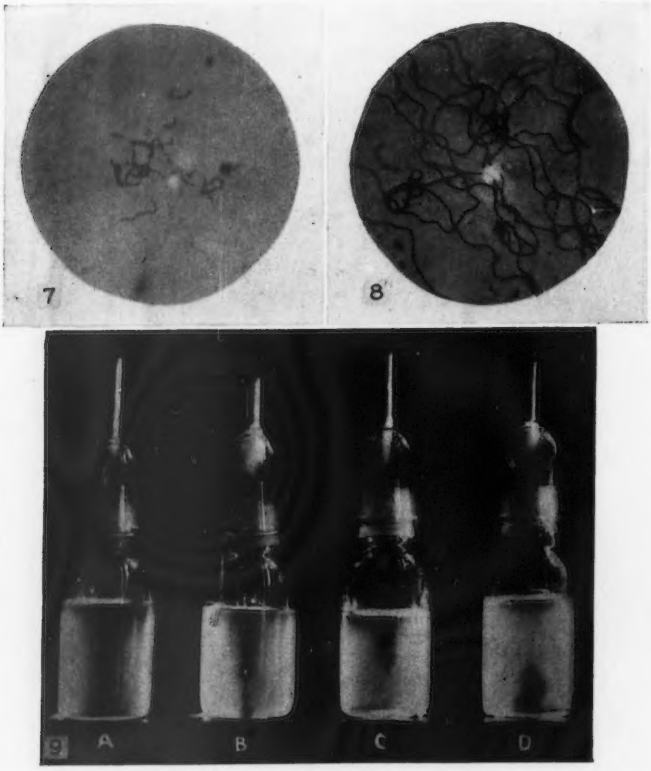
The change in morphology is always accompanied by a characteristic change in type of growth in liquid media. It has been pointed out by other workers in the field of microbic dissociation that occurrence of agglutination

in broth is associated with the rough type of colony (1, 3, 6, 12, 13, 14). All the organisms studied produced a homogeneous cloudy growth in broth when in the *S*-form, and a mucoid, gelatinous growth suspended in the clear supernatant medium when in the *R*-form.



FIGS. 1–6. *All colonies were grown on peptonized milk gelatin.* ×*35.* FIG. 1. *Strep. cremoris 114, smooth colony.* FIG. 2. *Strep. cremoris 114, lobate colony.* FIG. 3. *Strep. cremoris 114, aged colony showing outbursts.* FIG. 4. *Strep. cremoris 114, aged colony showing formation of daughter colonies.* FIG. 5. *Strep. cremoris 114, rough colony.* FIG. 6. *Strep. cremoris 142, rough colony.*

A difference in the type of growth may also be observed in agar stab cultures. A pure smooth strain produces an even filiform growth along the line of inoculation (Fig. 9a). At some stages of growth, outbursts from several points in the stab are formed (Fig. 9b). These outbursts are probably the same type of transition growth that is formed in colonies aging on gelatin plates. The true *R*-culture gives a very fuzzy arborescent growth in agar stab culture. The majority of strains studied seem to be more aerobic in this condition than in the *S*-form, the growth terminating at a point approximately half-way down the stab (Fig. 9c). The exception to this is *Strep. cremoris* 114. This organism, when in the rough stage, usually grows only at the bottom of the tube (Fig. 9d).



FIGS. 7–9. FIG. 7. *Strep. cremoris 142, smooth strain; 48-hr. broth culture. Gram stain. ×1100.* FIG. 8. *Strep. cremoris 142, rough strain; 48-hr. broth culture. Gram stain. ×1100.* FIG. 9. *Three-week-old agar stab cultures. (a) Strep. lactis 232, smooth strain. (b) Strep. lactis 232, agar stab showing outbursts. (c) Strep. lactis 232, rough strain. (d) Strep. cremoris 114, rough strain.*

Biochemical Characters

The formation of a true *R*-dissociant results in the complete loss by the organism of the ability to ferment lactose, either in milk or in broth. This biochemical variation sometimes occurs prior to the onset of dissociation as revealed by colonial and cultural characters. When this phenomenon occurred, the cultural and colonial changes associated with dissociation usually developed in the course of a few transfers. Although some evidence has been obtained that there is a tendency for the *R*-form to revert to the colonial characters of the *S*-form, a complete reversion has not as yet been accomplished, and on no occasion has a rough form ever acquired lactose-fermenting ability. In confirmation of previous findings (11), the inhibition of lactose-fermenting ability is not accompanied by a failure to ferment the monosaccharide glucose. The *R*-variant continues to ferment glucose with a vigour equal to that possessed by the *S*-form.

The induction of dissociation and the accompanying inhibition of lactose-fermenting ability took place most readily in peptonized milk broth containing 2% glucose. The addition of 0.25% lithium chloride or of 0.1% phenol to either peptonized milk broth or casein digest broth, failed to induce dissociation. A similar finding in the case of *Streptobacterium plantarum* has been described by Tracy (15). In contradiction of the results obtained by Tracy and those reported upon herein, Staryghina (13) was able to induce microbic dissociation in the case of both lactic acid streptococci and streptobacteria by transfer in lithium chloride or phenol broths.

Susceptibility to Dissociation

The number of transfers required to bring about microbic dissociation varied not only for different streptococci, but also for the same culture grown under similar conditions on different occasions. This inconsistency in the behaviour of the lactic acid streptococci, which has also been described by Whitehead and Cox (16), was especially marked in the case of *Strep. lactis* 146.

At first this organism dissociated very readily, rough colonies making their appearance at approximately the twentieth transfer. The rough culture continued to show abundant growth in the medium for four subsequent transfers. The fifth glucose peptonized milk broth tube, however, remained apparently sterile. No colonies developed on poured plates made from this broth tube.

Based on the assumption that the sudden cessation of obvious growth following closely upon a change from the *S*- to *R*-type was analogous to the development of a *G*-form as described by Hadley, Delves and Klimek (7), Duff (2), Kopeloff (9) and others, in several different types of micro-organisms, the Hauduroy (8) "washed plate" technique was applied to the apparently sterile broth tube in an attempt to demonstrate the existence of this form of the lactic acid streptococci. Although the washed plate series was carried to the sixth plating, no growth was observed, and consequently the series was discontinued.

Repetition of the work described above gave similar results as far as the sequence of dissociative changes was concerned, but the change from the *S*- to the *R*-type, with subsequent failure to grow, took place much more rapidly.

The phenomenon of dissociation was not encountered on repetition of this experiment at a later date. Even after 60 transfers in glucose peptonized milk broth, followed by 15 serial transfers in casein digest broth to which 0.1% phenol had been added, no obvious change in any of the characters of the organism was observed. The colony form remained round and smooth, the growth in broth was always cloudy, and there was no evidence of loss of lactose-fermenting ability.

Of the cultures studied, *Strep. lactis* 146 was the only strain found to exhibit the phenomenon of sudden cessation of growth on serial transfers of the *R*-forms in broth. In all other strains, a stable *R*-form appeared on dissociation.

It is evident that the phenomenon of dissociation in the lactic acid streptococci is erratic in its appearance, and it would appear to depend on the sensitivity of the organism to the experimental conditions employed for the induction of dissociation. Microbic dissociation will not occur unless the micro-organism is in a susceptible condition. It has not been possible as yet to determine the factors responsible for the change of an organism from a stable smooth form to a smooth form susceptible to variation.

Conclusions

The results of the work reported upon herein clearly show that microbic dissociation of lactic acid streptococci may be induced by the presence in the medium of a carbohydrate with a specific configuration. It is suggested that the micro-organism must be in a susceptible condition before dissociation can occur. The variations in cultural and colonial characters are not constant for different strains of the same species, nor does the same strain follow a definite sequence of changes on dissociation at different times. The variations that occur among the lactic acid streptococci on dissociation are similar to those described for other bacterial species.

Acknowledgment

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VEGETATIVE PROPAGATION OF CONIFERS

I. ROOTING OF CUTTINGS TAKEN FROM THE UPPER AND LOWER REGIONS OF A NORWAY SPRUCE TREE[1]

By N. H. Grace[2]

Abstract

Cuttings from the upper and lower regions of a Norway spruce tree were treated with talc only, and with talc containing 1000 p.p.m. indolylacetic acid. Ten weeks after being planted in sand, 43% of the upper and 75% of the lower cuttings were rooted. Hormone treatment increased the number of roots per rooted cutting but decreased the mean length of root. Lower cuttings produced twice the length of root of the upper cuttings, and the mean length of individual roots was also significantly greater. Nineteen weeks after being planted, the cuttings not rooted at 10 weeks were re-inspected, and gave final rooting values for the experiment of 48% for upper cuttings and 86% for lower. Physiological differences are consequently suggested in cuttings taken from the upper and lower regions.

Vegetative propagation of conifers is essential to rapid improvement through forest tree breeding, because valuable parental stock and hybrids could be multiplied and tested by this means; but comparatively little is known concerning the factors affecting successful propagation of cuttings from many varieties of tree. The position of the cutting on the tree is one of the factors to be considered. It is possible that there are physiological differences in the rooting response of cuttings taken from the upper and lower regions of the spruce, since the upper region bears the female flowers, the lower the male, and the wide-spreading lower branches of some varieties have a tendency to layer. Accordingly, a small preliminary experiment now to be described was carried out in the greenhouse of the National Research Laboratories in order to investigate this point.

Experimental

In mid-November 1938, branches were collected from a typical Norway spruce (*Picea excelsa*) tree, approximately 18 years of age and situated in a plantation at the Dominion Forest Station, Chalk River, Ontario. The branches, taken from what were roughly the upper and lower third portions of the tree, carried current year's growth of approximately the same length. The bases of the branches were packed in moist peat, and the package left outside in this manner until mid-January 1939. The dormant material was then divided into full-length cuttings, which were torn off at the node; jagged edges were trimmed with a knife, below the heel. There were 90 cuttings from each position, comprising terminals and laterals in the approximate ratio of one terminal to four laterals. Cuttings ranged from 2 to 4 in. in

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[2] *Biochemist, National Research Laboratories, Ottawa.*

length, and were divided into groups of 15, each group being representative of the different lengths. Prior to planting, three replicates of 15 cuttings from each position were dusted with talc only, and three with talc containing 1000 p.p.m. of indolylacetic acid. Random arrangement in the propagation frame permitted statistical analysis of the results for differences due either to position or to chemical treatment.

The cuttings were planted in washed brown sand in a cotton-covered propagation frame placed in the greenhouse and equipped with electrical bottom heat cables. The room temperature ranged around 65° F., while the sand temperature was held at approximately 72° F. The cuttings were held in this frame for 10 weeks, when they were dug up for observation; the unrooted cuttings were then replaced in the frame for a further period of 9 weeks, when final observation was made.

Results

Record was made of the number of cuttings rooted in each replicate of 15; further, the number of roots and their length were determined and expressed as number of roots and millimetres of root per rooted cutting. Finally, the mean root length was calculated. These four sets of data were subjected to analyses of variance to bring out the effects of position of the cutting on the tree, treatment with 1000 p.p.m. indolylacetic acid, and the interaction between position and treatment.

The results of the four mathematical treatments of rooting data are given in Table I. It is apparent that cuttings from the lower part of the tree rooted substantially better than those taken from the upper region. Lower cuttings produced more than double the total length of roots of the upper cuttings and the individual roots are appreciably longer. However, position had no effect on the number of roots produced. While there is an apparent difference of 12% in rooting in favour of the treatment with 1000 p.p.m. indolylacetic acid, this is not statistically significant. Treatment with hor-

TABLE I

ROOTING OF NORWAY SPRUCE CUTTINGS 10 WEEKS AFTER PLANTING

	Source of cuttings		Treatment		Necessary difference, 5% level
	Upper	Lower	Talc only	1000 p.p.m. indolylacetic	
Cuttings, rooted, transformed data*	2.7	3.4	2.9	3.2	0.58
Cuttings rooted, %	43	75	53	65	
Number of roots per rooted cutting	1.9	2.2	1.7	2.4	0.67
Total root length per rooted cutting (mm.)	15.2	31.9	22.9	24.2	11.7
Mean root length (mm.)	8.6	14.6	13.1	10.1	3.0

* *Data transformed to* $\sqrt{x + \frac{1}{2}}$ *basis (1).*

mone, however, increases significantly the number of roots per rooted cutting; it has no significant effect on the total root length, but reduces significantly the length of individual roots. The last is an observation frequently encountered when root lengths are considered. None of the interactions between treatment and position attained significance.

After a further period of 9 weeks, *i.e.*, 19 weeks in all from the date of planting, the cuttings not rooted at 10 weeks were again examined. The 19-week totals indicated that upper cuttings rooted 48%, lower, 86%; cuttings treated with talc only, 60%, with talc containing 1000 p.p.m. indolylacetic acid, 73%. Analysis of variance showed the effect of position on the tree significant to the 1% level; hormone effects failed to reach significance.

While these results deal with dormant cuttings from only one tree, they do indicate physiological differences in branches from the upper and lower parts of this individual. Further experiments on a number of trees, and extension to other species of spruce, are required to establish the generality of this condition. It will be necessary also to consider the growth of rooted cuttings from these different regions, for it is essential that normal trees be produced; increased ease of rooting from cuttings taken from lower branches will be of no practical value unless such cuttings grow normally and produce properly formed trees.

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FLAX STUDIES

IV. THE PHYSICAL AND CHEMICAL CHARACTERISTICS OF FLAXSEED AT PROGRESSIVE STAGES OF MATURITY[1]

By F. H. Lehberg[2], W. G. McGregor,[3] and W. F. Geddes[4]

Abstract

Flaxseed from plots seeded at Ottawa and Saskatoon over a period of several weeks and harvested at maturity showed no difference in oil content with dates of seeding, but the iodine values tended to increase with late seeding. Experiments conducted with Bison and Redwing grown in 1936 at Brandon, Saskatoon, Edmonton, and Fallis, and with Redwing in 1937 at Brandon, Edmonton, and Fallis, in which the seed was harvested at successive stages of maturity, showed that moisture decreased and dry kernel weight and oil content increased with progressive maturity up to approximately thirty days after flowering. Rate of oil deposition was in some cases extremely rapid, from 80% to 90% of the maximum oil found being deposited by the fifteenth to eighteenth day. Oil content and dry kernel weight reach a maximum several days before visual maturity. Unsaturation proceeds somewhat more slowly and reaches a higher value under climatic conditions favouring slow maturity.

Introduction

In Western Canada, the northern limit of flaxseed production is conditioned by the time required to reach maturity, and it is frequently necessary to harvest the crop before it is fully ripened in order to avoid possible damage from frost. As immaturity is a degrading factor, it is of importance to secure definite information as to its effect on oil content and the drying value of the oil under Canadian cultural conditions.

In other countries some attention has been given to this subject, but only a few investigators have studied progressively the changes in chemical composition as the seed matures; there is little information on the development of unsaturation. Ivanow (9) showed that the oil content of flaxseed harvested at four stages of growth increased with maturity, the values in one year ranging from approximately 4.5% one week after flowering to 35.0% seven weeks later, at which time the seeds were fully ripe. Eyre and Fisher (7) of England reported a range from 21 to 40.9% between the oil content of quite green and ripe seeds separated from plants having green and ripe bolls. On the other hand, at the North Dakota Experimental Station, Washburn (12) found an extreme range of only 38.8 to 40.8% oil for green and mature seeds. Coleman and Fellows (4) reported that immature seeds separated from a number of bulk samples contained an average of 32.6%

[1] *Manuscript received April 1, 1939.*

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oil as compared with 41.4% for the mature seeds. At the South Dakota Experiment Station, Bushey, Puhr, and Hume (3) determined the oil content of flaxseed harvested at five different dates of maturity in 1926 and found it to increase from 29.5%, when approximately one-third of the seeds were greenish in colour, to 36.8% at full maturity, about four weeks later. Dillman (5) carried out a detailed study of the daily seed growth and time of oil formation in the developing seed from flowering to maturity in Minnesota and North Dakota during the years 1926 and 1927; his data show that the most rapid formation of oil occurs between the seventh and eighteenth days after flowering and is correlated with the increase in dry weight. The maximum dry weight and oil content are reached from six to nine days before the seeds are fully ripe, after which there is little change to full maturity. Johnson (10) studied the rate of development of the oil on soils of different productivity in Minnesota. The average oil content of Bison flax showed a fairly uniform increase up to 25 days after flowering, followed by a slight decrease to maturity. The iodine number increased rapidly to 17 days after flowering, remained constant for a short period, then decreased slightly to complete maturity. In the studies reported by Johnson (10), and also by Dillman (5), a large number of flowers were marked and the bolls harvested at a definite period in their development to obtain seed of uniform and definite age. Delayed planting produced a slight reduction in oil percentage during one season but no consistent difference during the other season studied. The iodine number of the oil indicated a slight decrease with delayed planting for Bison but no change for Redwing. In both varieties studied the iodine number at the last date of planting was high.

Under the conditions of the north of Ireland, Eyre (6) and Barker (2) made a study of the development of oil in the seeds of the flax fibre crop with the variety commercially known as "Dutch Blue". They found that the synthesis of the oil takes place slowly up to the eleventh day, after which a period of rapid accumulation sets in, amounting to about 3% per day for some 10 days, when, about 21 days after flowering time, the maximum oil content is reached. The iodine value increased gradually throughout the whole period, the greatest rate of increase taking place after the oil content had reached its maximum value. Robinson (11), working in Michigan with a fibre variety "Saginaw", also found that the oil content of the seed increased with maturity, reaching a maximum before fully ripe.

The study here reported was initiated by the Sub-Committee on Oil Seeds, Associate Committee on Grain Research, and Dominion Department of Agriculture, with the object of investigating the progressive changes in the chemical composition of maturing flaxseed with particular reference to oil content and degree of unsaturation, as measured by iodine value. The co-operating organizations included the Cereal Division, Dominion Department of Agriculture, Ottawa; Grain Research Laboratory, Board of Grain Commissioners, Winnipeg; Department of Agronomy, University of Saskatchewan, Saskatoon; and the Department of Field Crops, University of Alberta, Edmonton.

Experimental

The investigations were begun in 1934 and extended over four crop years. In 1934 and 1935 a "dates of seeding" experiment was conducted in which the bolls were harvested when mature, while in the following two years a study was made of the changes in chemical composition of the seed at progressive stages of maturity.

In all, three varieties, Bison, Redwing, and Novelty, which are the most extensively grown in Canada for seed production, were represented. Bison is characterized by a tall vigorous growth habit, is somewhat late in maturity, and yields medium large seeds. Redwing produces a shorter, stronger stem, matures approximately 10 days earlier, and yields smaller seeds than Bison. Bison has a higher oil content with a lower iodine value than Redwing grown under similar conditions. Novelty is intermediate between Bison and Redwing as regards maturity and size of seed.

For the "dates of seeding" experiment in 1934, Bison, Redwing, and Novelty were seeded at Ottawa, Ontario, in quadruplicate plots, arranged systematically, and trimmed to a rod-row length at harvest time. Seeding commenced as early as land could be prepared and was continued at five-day intervals until ten seedings were made. In 1935, a similar experiment was carried out at Saskatoon in which the variety Bison was sown in duplicate plots at five different dates.

For the study of the physical and chemical characteristics of flaxseed at different stages of maturity in 1936, Bison and Redwing were grown as a quadruplicated, randomized, two-variety, rod-row test at Brandon (Manitoba), Saskatoon (Saskatchewan), and Edmonton and Fallis (Alberta). Sufficient rod-rows were seeded in each plot to provide one for sampling at each stage of maturity.

Harvesting of the bolls commenced at the 15th day after approximately 75% of the plants were in blossom and was continued at three-day intervals until the 36th day after flowering, at all the stations except Fallis, where for practical reasons collections were made at approximately six-day intervals. At each sampling all the bolls from one rod-row were collected, so that there was some variation in the stage of development, because the flax plant continues to flower day by day over a period of 7 to 10 days. In the 1937 experiment, Redwing only was seeded at the same stations, and harvesting was begun at 6 rather than 15 days after 75% of the plants were in blossom. Because of drought, no samples were available from Saskatoon in 1937. In both years, quadruplicate samples of each collection were taken and bulked for laboratory testing.

The bolls harvested at the different stations were forwarded in air-tight containers to Winnipeg for analysis. The bulk of each sample was dried *in vacuo* for 14 hr. at 100° C. without weighing, a small sub-sample being retained for a moisture determination in order that the changes in moisture content with maturity might be followed. Moisture was determined, on

approximately 1 gm. of the whole kernels obtained from the original subsample set aside for the purpose, by drying in a vacuum oven at 100° C. for 24 hr. As some of the high-moisture samples in 1936 had to be discarded on account of spoilage during shipment, and also in order to reduce the possibility of respiration causing changes in chemical composition, the main portion of each sample from the more distant stations (Edmonton and Fallis) in 1937 was dried for a few hours at 100° C. under partial vacuum prior to shipment; but a portion of the freshly harvested material was forwarded in a sealed container for moisture determinations.

The partially dried bulk samples were hand-threshed, and the seed was ground to a fine pulp with a mortar and pestle; the moisture content was determined by the vacuum-oven method, and the dried residue employed for other analytical determinations. Oil content was determined on from 2 to 10 gm. (depending on the stage of maturity) of the previously dried and ground material, by extracting with anhydrous alcohol-free and peroxide-free ethyl ether in a Soxhlet extractor, with use of Whatman double thickness extraction thimbles. After 4 hr. of extraction, the samples were re-ground in a mortar with reagent sea-sand and re-extracted for a further 16 hr. at a siphoning rate of one per minute. To remove traces of starch, the ether extract was filtered through a sintered glass filter and transferred directly to a tared 125 cc. Erlenmeyer flask by suction, and the extraction flask was washed three times with fresh solvent. The excess ether was distilled off on a water bath maintained at approximately 70° C., the extract dried *in vacuo* for 3 hr. at 98° to 100° C. at a pressure not exceeding 25 mm. mercury, and weighed. The oil content was expressed on a dry matter basis. This extraction procedure for determining the oil content was employed in this study rather than the rapid refractometric method described by Geddes and Lehberg (8), as variations in the refractive indices of linseed oil extracted from flaxseed of varying maturity would lead to relatively large errors by the latter method.

In 1934 and 1935, iodine values were determined on cold-pressed oil obtained by means of the Carver Laboratory hydraulic press; but owing to the limited material available in the maturity studies carried out in 1936 and 1937, it was necessary to utilize the ethyl-ether extracts. Wijs' method, as detailed by the American Association of Official Agricultural Chemists (1), was used in all cases. Nitrogen was determined on a one-gram sample by the Kjeldahl-Gunning-Arnold procedure essentially as described by the A.O.A.C. All analyses were conducted in duplicate.

Relation Between Date of Planting and Flaxseed Quality

1934 and 1935 Experiments

The results of the dates of seeding experiments, summarized in Table I, reveal no definite trend in oil content, but there is a definite tendency for the iodine values to increase with delayed seeding, probably because of the cooler weather prevalent during the maturation of the late-seeded plots.

TABLE I

OIL CONTENT AND IODINE VALUE OF MATURE FLAX FROM PLOTS SEEDED AT DIFFERENT DATES

Variety	Date seeded	Days to maturity	Height, in.	Yield per acre, bu.	Grade	Oil content (dry basis), %	Iodine value of cold pressed oil (Wijs')
Ottawa—1934							
Bison	4 May	86.0	22.0	24.9	1 C.W.	42.3	180.8
	9	84.0	23.0	25.7	1 C.W.	42.6	180.8
	14	83.5	23.0	24.1	1 C.W.	41.7	180.8
	19	83.0	23.7	26.1	1 C.W.	41.1	181.1
	24	82.0	24.2	24.4	1 C.W.	41.1	182.6
	29	84.7	22.7	17.3	1 C.W.	41.2	183.3
	2 June	89.0	21.5	10.5	1 C.W.	41.1	—
	8	89.0	21.0	10.9	1 C.W.	41.1	183.8
	13	97.0	21.2	11.9	1 C.W.	41.5	184.0
	18	92.0	22.7	8.0	1 C.W.	40.7	184.0
Redwing	4 May	82.5	20.5	20.2	1 C.W.	40.3	—
	9	80.5	20.3	20.1	1 C.W.	41.4	189.8
	14	78.2	21.7	18.9	1 C.W.	40.7	190.1
	19	75.7	22.0	21.4	1 C..W	40.6	190.8
	24	74.7	22.7	20.1	1 C.W.	40.7	190.8
	29	76.2	22.5	16.7	1 C.W.	40.7	191.6
	2 June	78.0	19.7	15.8	1 C.W.	40.3	193.5
	8	76.0	19.5	14.1	1 C.W.	40.3	193.5
	13	79.0	17.5	8.0	1 C.W.	40.2	193.9
	18	79.0	19.5	—	1 C.W.	39.9	—
Novelty	4 May	85.0	20.7	22.7	1 C.W.	42.9	182.6
	9	82.5	20.7	23.1	1 C.W.	42.1	183.1
	14	83.2	21.2	—	1 C.W.	41.0	183.1
	19	81.2	21.2	25.4	1 C.W.	41.0	184.6
	24	82.0	21.2	22.0	1 C.W.	41.1	184.7
	29	85.2	20.7	17.4	1 C.W.	41.2	184.9
	2 June	89.0	17.0	—	1 C.W.	40.7	184.9
	8	89.0	17.0	—	1 C.W.	40.9	185.6
	13	97.0	19.0	9.2	1 C.W.	41.8	188.0
	18	92.0	19.7	8.2	1 C.W.	42.5	186.5
Saskatoon—1935							
Bison	24 April	125.0		12.8	1 C.W.	42.6	180.6
	3 May	116.0		15.8	1 C.W.	42.2	183.8
	15	110.0		21.2	1 C.W.	42.9	187.8
	1 June	96.0		8.0	1 C.W.	43.0	190.8
	19	Not mature		8.9	1 C.W.	42.0	192.0

In this connection it is interesting to note that the late-harvested samples of Bison from Saskatoon were higher in iodine number than those from Ottawa, where the average temperature during the late summer is higher.

All varieties made a more vigorous growth when seeded about two weeks after the season opened. Delayed seeding appears to increase the number of days to maturity for Bison and Novelty, the inverse being true for Redwing.

TABLE II

PHYSICAL AND CHEMICAL CHARACTERISTICS OF FLAXSEED AT PROGRESSIVE STAGES OF MATURITY, 1936 EXPERIMENTS

Days after flowering	Date harvested		Number of days from seeding to harvesting		Appearance		Moisture, %		Weight per 100 kernels (dry basis), gm.		Oil content (dry basis), %		Iodine value (Wijs')	
	Bison	Redwing	Bison	Redwing	Bison	Redwing	Bison	Red-wing	Bison	Red-wing	Bison	Red-wing	Bison	Red-wing
Brandon (seeded May 18)														
15	July 21	July 15	64	58	Green	Green	63.0	65.0	0.33	0.17	32.9	33.0	136.8	139.9
18	24	18	67	61	Green	Green	67.2	69.6	0.38	0.25	34.7	35.1	165.3	161.7
21	27	21	70	64	Brownish	Green	65.2	64.7	0.46	0.32	37.6	37.1	163.3	159.5
24	30	24	73	67	Brown and immature	Brown and immature	45.2	53.4	0.48	0.33	36.5	37.0	169.1	173.1
27	Aug. 2	27	76	70	Immature	Brown and immature	21.0	22.3	0.50	0.35	39.8	37.7	168.4	174.0
30	5	30	79	73	Immature	Immature	10.6	12.2	0.53	0.37	38.2	37.0	172.8	174.6
33	8	Aug. 2	82	76	Nearly mature	Mature	7.7	8.1	0.54	0.39	38.0	37.3	175.3	178.7
36	11	5	85	79	Nearly mature	Mature	7.6	7.8	0.54	0.39	38.6	37.8	173.8	179.3
Saskatoon (seeded May 13)														
15	July 18–19	July 18	66.5	66	Green	Green	67.3	65.3	0.22	0.22	35.0	35.4	130.2	142.4
18	21–22	21	69.5	69	Green	Green and brown	55.2	52.7	0.27	0.29	36.3	36.2	159.6	165.0
21	24–25	24	72.5	72	Green and immature	Brown and immature	28.5	26.8	0.33	0.36	36.8	36.2	182.4	182.4
24	27–28	27	75.5	75	Immature	Brown and immature	10.6	10.9	0.38	0.39	38.2	38.0	180.7	181.9
27	30–31	30	78.5	78	Immature	Brown and immature	10.2	9.8	0.39	0.40	38.6	39.0	182.2	187.4
30	Aug. 2–3	Aug. 2	81.5	81	Mature	Fully mature	7.6	7.8	0.39	0.40	39.8	39.0	181.8	182.7
33	5–6	5	84.5	84	Mature	Fully mature	6.6	—	0.39	0.40	39.4	38.2	185.5	185.6
36	8–9	8	87.5	87	Mature	Fully mature	6.8	—	0.40	0.40	39.4	37.8	186.1	186.8

TABLE II—*Concluded*

PHYSICAL AND CHEMICAL CHARACTERISTICS OF FLAXSEED AT PROGRESSIVE STAGES OF MATURITY, 1936 EXPERIMENTS—*Concluded*

Days after flowering	Date harvested		Number of days from seeding to harvesting		Appearance		Moisture, %		Weight per 100 kernels (dry basis), gm.		Oil content (dry basis), %		Iodine value (Wijs')	
	Bison	Redwing	Bison	Redwing	Bison	Redwing	Bison	Red-wing	Bison	Red-wing	Bison	Red-wing	Bison	Red-wing
Edmonton (seeded May 12)														
15	July 16–17	July 13–14	65–66	62–63	Green	Green	79.0	76.4	0.16	0.13	11.1	11.4	125.4	122.9
18	19–20	16–17	68–69	65–66	Green	Green	78.1	76.5	0.22	0.17	18.4	20.5	136.0	139.7
21	22–23	19–20	71–72	68–69	Green	Green	72.8	73.5	0.25	0.23	33.1	33.8	144.4	145.5
24	26	22–23	75	71–72	Green	Green	73.0	71.2	0.33	0.29	36.4	36.9	153.8	159.3
27	28–29	26	77–78	75	Green	Green and brown	67.0	71.7	0.41	0.37	40.4	38.8	162.6	169.6
30	31, Aug. 1	28–29	80–81	77–78	Green and brown	Brown and immature	—*	62.2	0.53	0.41	41.1	39.4	180.4	182.4
33	Aug. 3–4	31, Aug. 1	83–84	81	Brown and immature	Brown and immature	52.0	48.3	0.53	0.42	41.8	40.3	182.7	185.5
36	7	Aug. 3–4	87	83–84	Brown and immature	Brown and immature	56.6	42.7	0.52	0.43	41.9	40.3	184.3	187.8
Fallis (seeded May 19)														
15	July 26–27–28	July 21–22	68–69–70	63–64	Green	Green	77.7	79.3	0.15	0.14	20.1	21.8	—*	—*
21	Aug. 1–2	27–28	74–75	69–70	Green	Green	75.2	74.8	0.31	0.26	29.9	31.6	—*	—*
27	7–8	Aug. 3	80–81	76	Green	Green	74.5	—*	0.38	0.29	32.7	35.2	—*	—*
33	14	8–9–10	84–87	81–82–83	Green	Green and brown	73.2	40.5	0.53	0.33	41.4	39.4	—*	—*
39	19–20	15–16	92–93	88–89	Green and brown	Green and brown	70.0	52.6	0.58	0.41	41.5	38.7	—*	—*
45	26–27	20–21	99–100	93–94	Green and brown	Immature	63.0	—*	0.58	0.44	41.3	40.2	—*	—*
51	31, Sept. 1–2	26–27–28	104–105–106	99–100–101	Green and brown	Immature	—*	49.2	—	0.45	42.0	—	—*	—*
57	Sept. 7–8	Sept. 2	111–112	106	Very immature	Immature	—*	—*	—	—	42.0	—	—*	—*

* Not tested because of slight spoilage.

Varieties which do not reach a certain stage of maturity before the end of the season are retarded by second growth. As previous work had shown, later seeding usually produces an appreciable decrease in yield.

Physical and Chemical Characteristics of Flaxseed at Progressive Stages of Maturity

1936 Experiments

The results of the 1936 experiments are recorded in Table II, and represented graphically for each station excepting Fallis in Figs. 1, 2, and 3. It will be observed that, as indicated by oil content, the plants grown at Brandon and Saskatoon were considerably matured prior to the 15th day after flowering, when the first samples were collected. At all stations, both

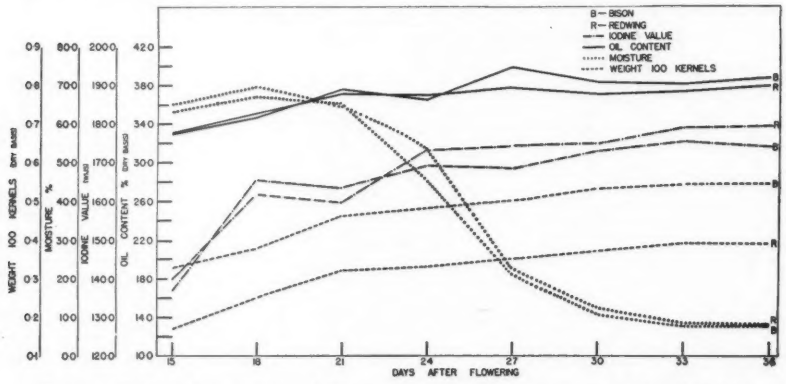

FIG. 1. *Various characteristics of Bison and Redwing flax grown at Brandon, Manitoba, in 1936 and harvested at different stages of maturity.*

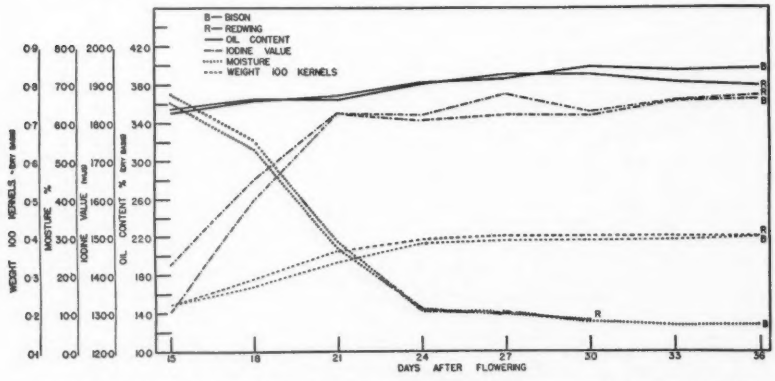

FIG. 2. *Various characteristics of Bison and Redwing flax grown at Saskatoon, Saskatchewan, in 1936 and harvested at different stages of maturity.*

varieties showed a decrease in moisture, increase in dry kernel weight and increase in oil content with progressive maturity up to approximately 30 days after flowering; the increase in iodine value continues after maximum dry kernel weight and oil content is attained.

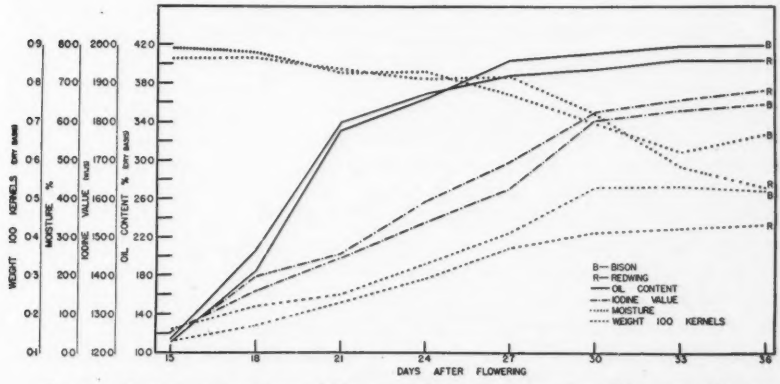

FIG. 3. *Various characteristics of Bison and Redwing flax grown at Edmonton, Alberta, in 1936 and harvested at different stages of maturity.*

These changes with maturity are more gradual for the Edmonton and Fallis series, and are probably associated with cooler weather and higher soil moisture at these points. The oil contents of the Edmonton samples were approximately 11% at the 15th day after flowering, increasing rapidly to 33% by the 21st day; as the oil content at the last harvesting was approximately 42%, 52% of the final oil was deposited within six days. After the 21st day the oil content increased more gradually and at the 27th day had reached 96% of the final value; at this time the kernels were still immature in appearance and had the characteristic greenish brown colour. The increase in iodine value at Edmonton is more gradual than the increase in oil content and continues throughout the entire collection period. At the 15th day the iodine values were 125.4 and 122.9 for Bison and Redwing respectively, increasing to 184.3 and 187.8 by the 36th day. At the final sampling the oil content of Bison was 1.6% higher than Redwing while the iodine value was 3.5 units lower.

At Brandon and Saskatoon, drier weather conditions prevailed and the days to maturity were less, than at Edmonton and Fallis. At the initial sampling approximately 85 to 90% of the oil finally found was already present, and the degree of unsaturation was also well advanced. For Brandon, the iodine values for Bison and Redwing increased from 136.8 and 139.9 units respectively at 15 days after flowering, to 173.8 and 179.3 units at 36 days, at which time the samples appeared mature.

At all stations, Bison yielded a higher final oil content and lower iodine value than Redwing; these are recognized varietal differences.

1937 Experiments

The analytical data for the 1937 experiments are summarized in Table III and graphically represented in Figs. 4, 5, and 6.

The general trends were similar to those observed in 1936; as maturity progressed moisture content rapidly decreased and the dry kernel weight, oil content, and iodine value increased. Oil deposition commenced very early in kernel development, approximately 17, 32, and 23% being present by the sixth day after flowering in the Brandon, Edmonton, and Fallis series respectively. It is extremely doubtful whether the estimation of the flowering stage was similar at the three stations, since from general climatic conditions one would expect the Edmonton and Fallis series to be lower in oil content than the Brandon series at this early state of maturity. This is again suggested by the comparative iodine values of 98, 129, and 120 units for the initial sampling at the above respective stations. As in 1936, the iodine values at all stations increased somewhat more slowly than oil deposition, reaching a maximum at a later stage of maturity. Visually, the Brandon, Edmonton, and Fallis samples were approaching maturity at 33, 40, and 54 days, respectively, after flowering. Complete maturity, as evidenced by the characteristic reddish-brown colour of Redwing, occurred on the 52nd and 58th days after flowering at Brandon and Edmonton, while the Fallis samples never reached full visual maturity; yet the iodine values for Fallis were the highest.

The fluctuations in the oil content of successive collections appear to follow the precipitation that occurred during the intervals between samplings, the oil content tending to increase after rain and to drop following a dry period. Iodine value does not appear to be similarly affected.

The nitrogen content of the seeds showed no pronounced trends with maturity in the Edmonton and Fallis series, but for Brandon there was a fairly consistent increase to the 24th day after flowering.

Discussion

The slight decrease in oil content and iodine number with delayed planting suggested by Johnson (10) was not apparent in these data. Oil content depends to some extent on maturity, and if all samples are mature not much variation in oil content is to be expected. However iodine number in each instance increased with delayed planting.

The results in regard to the rapidity with which oil is deposited in the developing kernel correspond quite well with those of other workers reported in the literature. Even at the sixth day after flowering there is an appreciable percentage of oil present, and in most cases approximately 80 to 85% of the maximum oil content found is present by the 15th to 18th day. The oil content increases with increasing dry kernel weight, both reaching a maximum several days before the sample is visually mature; this time varies with the environmental conditions. The data therefore indicate that harvesting the flax before it is completely ripe will not result in a decrease in oil yield.

TABLE III

PHYSICAL AND CHEMICAL CHARACTERISTICS OF REDWING FLAXSEED HARVESTED AT DIFFERENT STAGES OF MATURITY, 1937 EXPERIMENTS

Days after flowering	Date harvested	Number of days from seeding to harvesting	Appearance	Precipitation, in.			Moisture, %	Weight per 100 kernels, gm.	Oil content (dry basis), %	Iodine value (Wijs')	Nitrogen (dry basis), %
				1 day	2 days	3 days					
Brandon (seeded May 25)											
6	July 15	51	Green and very thin	—	—	.01	80.5	.12	17.0	98.3	2.93
9	18	54	Green and very thin	—	—	—	79.0	.15	23.9	120.4	3.23
12	21	57	Green and thin	—	—	.02	76.8	.20	30.4	126.3	3.83
15	24	60	Green and thin	.33	—	.09	72.5	.31	33.0	136.2	3.73
18	27	63	Green and plump	—	.08	—	64.8	.38	35.1	142.0	4.16
21	30	66	Green	—	.26	.02	64.5	.41	36.8	148.8	4.27
24	Aug. 2	69	Green	1.15	1.13	—	59.7	.46	41.3	152.0	4.47
27	5	72	Green	—	—	—	57.5	.45	38.4	176.0	4.40
30	8	75	Green and brown	—	—	1.17	60.2	.43	37.2	185.5	4.40
33	11	78	Immature and brown	.82	—	.11	55.3	.45	41.4	187.7	4.45
36	14	81	Immature and brown	—	Trace	.17	22.4	.49	40.1	189.2	4.50
39	17	84	Immature and brown	—	—	—	29.0	.46	38.8	184.6	4.45
42	20	87	Immature and brown	—	.29	—	44.0	.48	42.0	189.5	4.35
45	23	90	Immature and brown	—	—	—	22.2	.48	41.5	182.7	4.38
48	26	93	Immature and brown	—	—	—	16.4	.46	40.3	183.2	4.26
52	30	97	Brown, practically mature		Aug. 27, trace		17.8	.46	41.2	185.6	4.55
55	Sept. 2	100	Brown and mature	—	—	—	17.9	.46	40.6	186.3	4.26
58	5	103	Brown and mature	—	—	.30	14.5	.45	40.8	191.2	4.48
61	8	106	Brown and mature	—	—	—	10.0	.46	40.4	187.7	—
64	11	109	Brown and mature	—	—	—	8.0	.46	39.1	188.2	4.16
67	14	112	Brown and mature	—	—	—	5.6	.45	—	187.0	4.23
70	17	115	Brown and mature	—	—	—	5.4	.45	38.0	—	4.34
Edmonton											
6	July 24		Green and thin	.08	—	—	76.0	.21	32.0	128.8	3.69
9	27		Green and thin	—	.04	—	77.2	.29	35.2	139.0	3.70
12	30		Green and thin	.10	.30	.80	74.2	.28	37.6	162.3	3.73

TABLE III—*Concluded*

PHYSICAL AND CHEMICAL CHARACTERISTICS OF REDWING FLAXSEED HARVESTED AT DIFFERENT STAGES OF MATURITY, 1937 EXPERIMENTS—*Concluded*

Days after flowering	Date harvested	Number of days from seeding to harvesting	Appearance	Precipitation, in.			Moisture, %	Weight per 100 kernels, gm.	Oil content (dry basis), %	Iodine value (Wijs')	Nitrogen (dry basis), %
				1 day	2 days	3 days					
Edmonton—Concluded											
15	Aug. 2		Green and thin	.20	—	.01	72.3	.32	39.1	170.0	3.62
18	5		Green and thin	—	.06	.10	73.4	.33	37.5	182.0	4.05
21	8		Green	.04	—	.04	69.0	.38	40.3	187.0	4.11
24	11		Green	—	—	—	68.8	.40	40.8	196.0	4.08
28	15		Green	.12	.02	.24	65.2	.41	42.6	191.0	4.07
31	18		Green and brown	—	.01	.02	66.0	.38	39.7	189.5	3.75
34	21		Green	—	—	—	63.7	.41	43.3	190.0	3.92
37	24		Brown and some green	—	.18	—	61.8	.43	41.8	192.0	3.88
40	27		Immature and brown	—	.12	—	55.3	.48	41.2	185.0	4.06
43	30		Immature and brown	.02	Trace	—	61.9	.49	43.6	179.8	4.02
46	Sept. 2		Immature and brown	—	.12	.24	55.4	.44	41.6	180.2	4.05
49	5		Immature and brown	—	—	—	55.3	.49	42.7	195.5	4.16
52	8		Immature and brown	—	—	—	46.7	.50	42.4	197.5	3.83
55	11		Immature and brown	.04	—	—	42.0	.49	40.8	188.9	4.15
58	14		Practically mature	—	—	—	35.5	.52	41.5	184.5	4.18
65	17		Practically mature	—	—	—	35.0	.46	40.6	186.3	4.05
72	24		Practically mature	Sept. 20 .74	Sept. 21 .28	Sept. 22 .30	27.3	.48	42.2	189.8	3.55
Fallis											
6	July 30		Green	Not recorded			72.8	.26	23.6	120.4	3.61
12	Aug. 5		Green				69.8	.41	35.7	136.7	4.05
18	11		Green				76.0	.42	40.5	169.0	3.87
24	17		Green				70.0	.40	39.8	183.2	3.62
30	23		Green and brown				38.1	.40	39.0	194.6	3.35
36	29		Green and brown				66.4	.44	40.5	189.5	3.55
42	Sept. 3		Green and brown				46.6	.42	38.7	191.3	3.55
48	9		Green and brown				21.8	.45	40.6	199.3	—
54	15		Brown and immature				55.4	.44	40.0	196.5	3.28
61	22		Brown and immature				49.1	.46	39.3	194.0	3.94

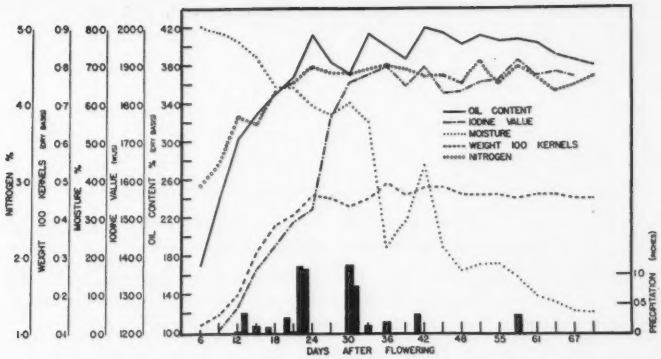

FIG. 4. *Various characteristics of Redwing flax grown at Brandon, Manitoba, in 1937 and harvested at different stages of maturity. Precipitation also shown.*

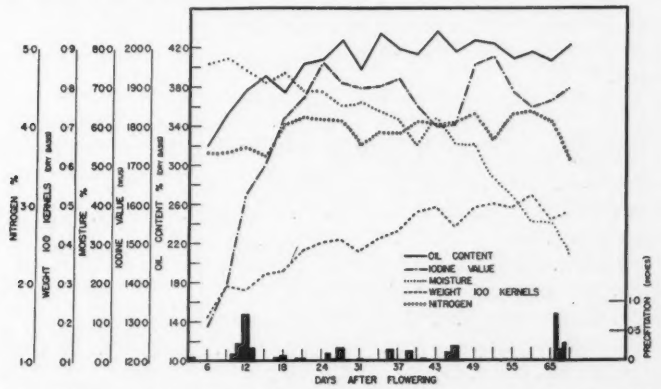

FIG. 5. *Various characteristics of Redwing flax grown at Edmonton, Alberta, in 1937 and harvested at different stages of maturity. Precipitation also shown.*

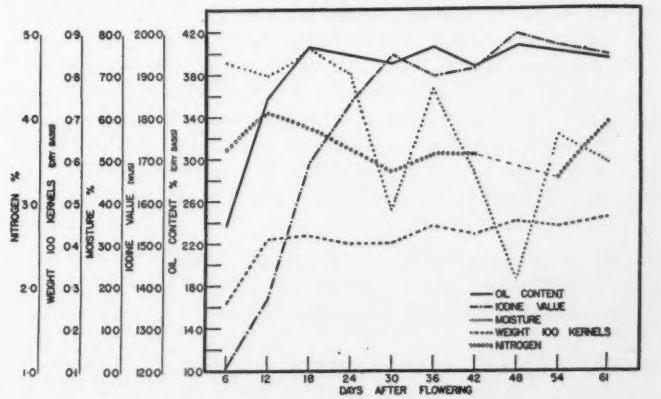

FIG. 6. *Various characteristics of Redwing flax grown at Fallis, Alberta, in 1937 and harvested at different stages of maturity.*

While there are some inevitable incongruities in the data, it is evident that the development of unsaturation proceeds somewhat more slowly than oil deposition and tends to reach a higher final value under climatic conditions favouring slow maturity. The results, however, are not sufficiently conclusive to warrant the statement that immaturity tends to result in lower iodine values, although the indications are in this direction.

The ability of flax to stand in the field beyond maturity without shattering is an important feature in harvesting operations, especially since the introduction of the combine harvester. Collections from the crop at Saskatoon in 1936 and at Brandon in both 1936 and 1937 would indicate that no deterioration in oil content or iodine number might be expected from this practice.

Acknowledgments

The authors are indebted to Dr. K. W. Neatby, University of Alberta, Dr. J. B. Harrington, University of Saskatchewan, and Mr. M. J. Tinline, Dominion Experimental Farm, Brandon, under whose direction the material was grown.

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SEPTORIA CANKER OF INTRODUCED AND NATIVE HYBRID POPLARS[1]

By J. E. Bier[2]

Abstract

Studies have shown that *Septoria musiva* Peck, a North American fungus which occurs commonly as a leaf-spotting parasite on native poplars, produces cankers, in addition to leaf injury, on certain introduced poplars (*Populus Rasumowskyana* Schneid., *P. Petrowskyana* Schneid., and *P. berolinensis* Dipp.), and the native hybrids, Northwest and Saskatchewan poplar.

Field observations and inoculation experiments demonstrated that most of the inoculum for spring infection arises from ascospores of a *Mycosphaerella* stage, and that the fungus enters the stems through mechanical wounds, uninjured lenticels, leaf petioles, or stipules. Incipient cankers occur in the bark of the current year's wood, soon girdling leading and side shoots. They later spread from lateral branches into the main stem, developing into perennial cankers which ultimately girdle and kill the trees.

Introduction

In recent years the attention of the author has been directed to a destructive canker of certain strains of poplar growing in the vicinity of Indian Head, Saskatchewan, and at the Petawawa Forest Experiment Station, Ontario. The disease was reported on *Populus Rasumowskyana* Schneid., *P. Petrowskyana* Schneid., *P. berolinensis* Dipp., Northwest poplar, and Saskatchewan poplar. The first three are introduced species of hybrid origin, which collectively have been called Russian poplar, while Northwest and Saskatchewan poplars are considered native hybrids between balsam poplar (*P. tacamahaca* Mill.) and cottonwood (*P. balsamifera* L.). Because of their hardiness, rapid growth, and ease of propagation, these species have been used extensively for windbreak and shelterbelt plantings in the Prairie Provinces.

When first observed it was noted that the cankers did not resemble those associated with *Hypoxylon*, *Cytospora*, or *Dothichiza*, the commonly recognized canker-producing fungi on poplar. Isolations from the bark of cankers of all ages repeatedly produced fruiting cultures of a species of *Septoria*. At this point in the investigation it was found that Mr. J. L. Van Camp had previously carried out experiments on the canker of Russian poplar, and had recorded a *Septoria* as the causal agent. A brief summary of Van Camp's work was published in the Report of the Director of Forestry for the year 1929–30 (1). These investigations were not continued after 1930, and no further research was carried out until 1936, when the problem was undertaken by this Service.

During the summer of 1938 the disease was investigated at Indian Head, Sask., and there is no doubt that the cankers and fungus studied in Ontario are the same as those previously described by Van Camp in Saskatchewan.

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The Disease

Historical Sketch of the Hosts and Disease

Russian poplars have been grown in Canada for the last 40 to 50 years. Saunders (7) in 1904, describing trees tested in Manitoba and the Northwest Territories, mentioned Russian poplar as a very hardy and fast-growing tree of value to the Northwest country. Johnson (2) in 1923, writing on tree planting in the Great Plains region, made the following statement with respect to Russian poplar: "It is hardy to winter cold and resists the ordinary amount of drought, but is subject to attack by canker, a disease which soon girdles the tree, generally near the base or at a crotch, and kills the portion above the canker. The disease is quite common on all Russian poplars grown in this region. For that reason they are not recommended for general planting."

In 1928, Munro (3) reported the presence of Russian poplar canker in Saskatchewan, and observed that the disease was more severe on higher planting sites. In 1930, Van Camp (1) recorded investigations on the disease which were carried out at Indian Head, Sask. A *Septoria*, identical with the fungus occurring as a leaf parasite on Russian poplar, was reported to be a wound parasite on the stems of *P. Petrowskyana* and Northwest poplar. Cankers were produced experimentally from wound inoculations made under greenhouse conditions, and methods were recorded for the control of the disease by the utilization of clean cuttings and clean planting sites. In 1933, Ross (6) advised against planting Russian poplars in large numbers on account of their susceptibility to canker and insect borers.

Canker Stage EXTENT OF DAMAGE

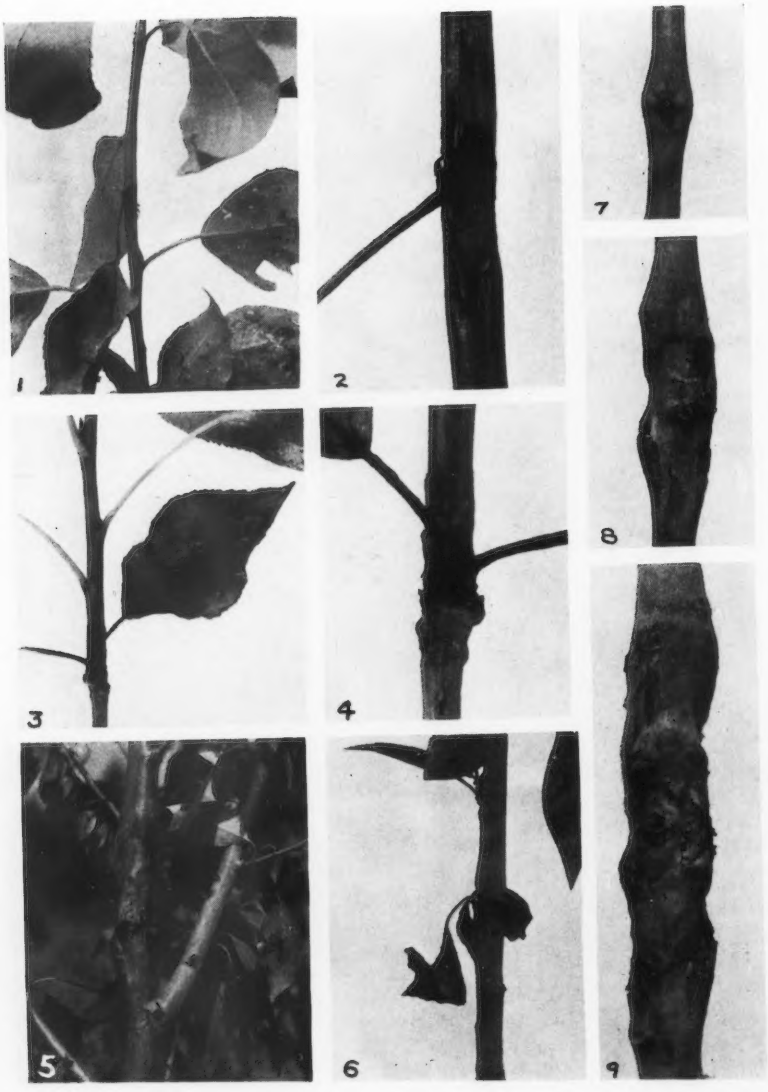
In Ontario, field observations on Septoria canker have been confined to a 13-year-old plantation of *P. Rasumowskyana* at the Petawawa Forest Experiment Station. On this area all trees are infected, each having a number of lesions on the trunk and branches which ultimately girdle and kill the affected parts. The average condition of the trees is illustrated in Plate I, which shows the upper half of a tree 17 ft. high. The host in turn forms new branches from adventitious buds on the stem, and frequently sucker shoots that may develop into trees are produced from the stumps. After 13 years of growth the trees are 10 to 20 ft. high, much branched, with from one to three stems arising from the original root system of each planted cutting.

Shelterbelts and plantations of *P. Petrowskyana*, Northwest, and Saskatchewan poplars in the prairie region of Manitoba, Saskatchewan, and Alberta have also been examined for cankers. The disease incidence coincided with the distribution of susceptible hosts, and on many occasions, large plantings established during the last 20 years had been practically eliminated as the result of cankers. In the vicinity of Edmonton, Alta., Russian poplars were in a much healthier condition than those observed in the southern part of the Prairie Provinces. Cankers were present, but the more vigorous growth of the trees resulted in the rapid production of secondary periderm around the lesions, which apparently was sufficient to inhibit the further advance of the pathogen.

PLATE I

Septoria canker on P. Rasumowskyana at the Petawawa Forest Experiment Station, Ont. The photograph shows the upper half of a 12-year-old tree which was approximately 17 ft. high. Note the two cankers on the main stem. The uppermost lesion has recently girdled the leader. The stem swelling immediately above the lower canker indicates a lesion on the opposite side of the trunk. Also observe the newly killed branch (the second from the ground on the right), and the responsible canker adjacent to the trunk. The leaves often persist on the dead parts until the following spring.

PLATE II



Leaf Spot Stage

Leaf lesions were universally present on trees susceptible to the canker stage (Plate IV). The injury was more severe on the leaves of lower branches, a condition possibly correlated with the discharge of ascospores from the perfect fruiting bodies produced on the overwintered leaves. Although no defoliation was noted as a direct result of leaf injury, the large amount of leaf area killed would appreciably reduce the photosynthetic activity, and in this way have a decidedly unfavourable effect upon the annual increment.

Disease Development

Most of the primary inoculum for the spring infection of leaves and stems arises from the abundant perithecia on the overwintered leaves. In addition to those on the ground, the perfect stage was found on leaves persisting on branches that had been killed during the previous growing season. Both perithecia and pycnidia containing viable spores were collected on lesions that had girdled the twigs developed during the previous year. Therefore, the spring dissemination of the pathogen is not wholly dependent on the ascospores. Ascospore discharge occurs from the time the buds open until the development of the new growth is complete.

On the Branches and Stems

Field observations and artificial inoculations have shown that cankers originate in the bark of twigs of the current year. The fungus may enter the host through mechanical wounds, uninjured lenticels, stipules, or leaf petioles (Plate III). By the middle of June under field conditions a very conspicuous symptom of the disease is the presence of one or more dead leaves on the leaders (Plate III, Fig. 1), at the ring scars (Plate II, Fig. 3), or on the axillary

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Plate II

Septoria canker on P. Rasumowskyana at the Petawawa Forest Experiment Station, Ont.

Fig. 1. *Stem canker arising at a leaf axil.* ×¾. *Note the dead leaf hanging down from the centre of the blackened diseased area.*

Fig. 2. *Enlargement of the lesion shown in Fig. 1.* ×2. *Note the pycnidia in the enclosed paler area of the lesion.*

Fig. 3. *Stem canker at the junction of the current and last year's growth.* ×¾. *Observe the small leaf on the left which was killed before reaching maturity.*

Fig. 4. *Enlargement of the lesion shown in Fig. 3.* ×2. *Note the blackened diseased area spreading into 2-year-old bark on the left.*

Fig. 5. *Canker on a 5-year-old stem.* ×¼. *Observe the swelling of the stem at the upper margin of the canker.*

Figs. 6 to 9. *Stages in the development of a perennial stem canker, arising from an initial infection in the current growth of an axillary shoot. The four lesions were collected from different positions on the same main stem.* ×½.

Fig. 6. *The current growth of an axillary shoot has become infected. Note the dead, shrivelled leaves on the black, dwarfed, axillary shoot which was produced during the second growing season of the stem. The infection has not as yet spread into the main stem.*

Fig. 7. *Canker condition on 3-year-old stems. The axillary shoot has fallen off, and the fungus has entered the main stem. Note the black, diseased bark below the branch scar, and the stem swelling around the diseased area.*

Fig. 8. *Canker condition on 4-year-old stems. Observe the pronounced stem swelling at the canker margins, and the spread of the infection into healthy bark at the top, central portion of the lesion.*

Fig. 9. *Canker condition on 5-year-old stems. Note the successive callous layers formed during the third and fourth growing seasons. The diseased bark at the top of the canker has been secondarily attacked by Cytospora. The lesion had just completed girdling the stem.*

branches produced on 2-year-old stems (Plate II, Fig. 6). Closer examination reveals stem lesions at the bases of the dead leaves (Plate II, Figs. 2 and 4). The diseased bark is usually black, frequently enclosing yellowish to white areas in which small pycnidia may be found (Plate II, Fig. 2, and Plate III, Fig. 2). The cankers girdle and kill the leader and axillary branches during the first growing season, but the spread of the disease from axillary branches into the main stem as a rule does not occur until the following year, *i.e.*, the third growing season of the stem (Plate II, Fig. 7). On entering the main stem, the pathogen produces a canker that is perennial in character. Each year considerable malformation of the stem arises at the margin of the lesion (Plate II, Figs. 8 and 9). In general, the cankers succeed in girdling the stems during their fourth or fifth year of growth.

Pycnidia and perithecia are found on infections on the current year's wood (Plate V, Fig. 1), but only on rare occasions have they been observed in cankers occurring on wood two or more years of age.

It is important to note that the bark of dead trees and cankered areas is secondarily attacked by *Cytospora chrysosperma* (Pers.) Fries (Plate II, Fig. 9), a fungus parasitic on some species of poplar. Isolations from the diseased bark at the margin of older lesions not infrequently produce cultures of *Cytospora*, and it is possible that advanced cankers may result from a combined attack of *Septoria* and *Cytospora*.

On the Leaves

The first leaf lesions appear from three to four weeks after the opening of the buds, and are usually confined to leaves on the lower branches. The number of lesions multiplies rapidly during the growing season, and the infection becomes more or less general throughout the trees. The fungus produces

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PLATE III

Cankers resulting from artificial inoculations.

FIG. 1. *Stem canker at a leaf axil of P. Rasumowskyana.* ×¾. *On April 22, 1938, spores were placed at approximately the mid-point on the leaf petiole. A petiole lesion developed and the fungus was observed to pass down the petiole into the stem. The photograph was taken on June 15.*

FIG. 2. *Stipule infection on Northwest poplar.* ×¾. *On June 6, 1938, spores were placed on the green stipules at the leaf axil. Lesions formed on the stipules, and stem cankers developed from their bases. Note the pycnidia on the canker. The photograph was taken on June 30.*

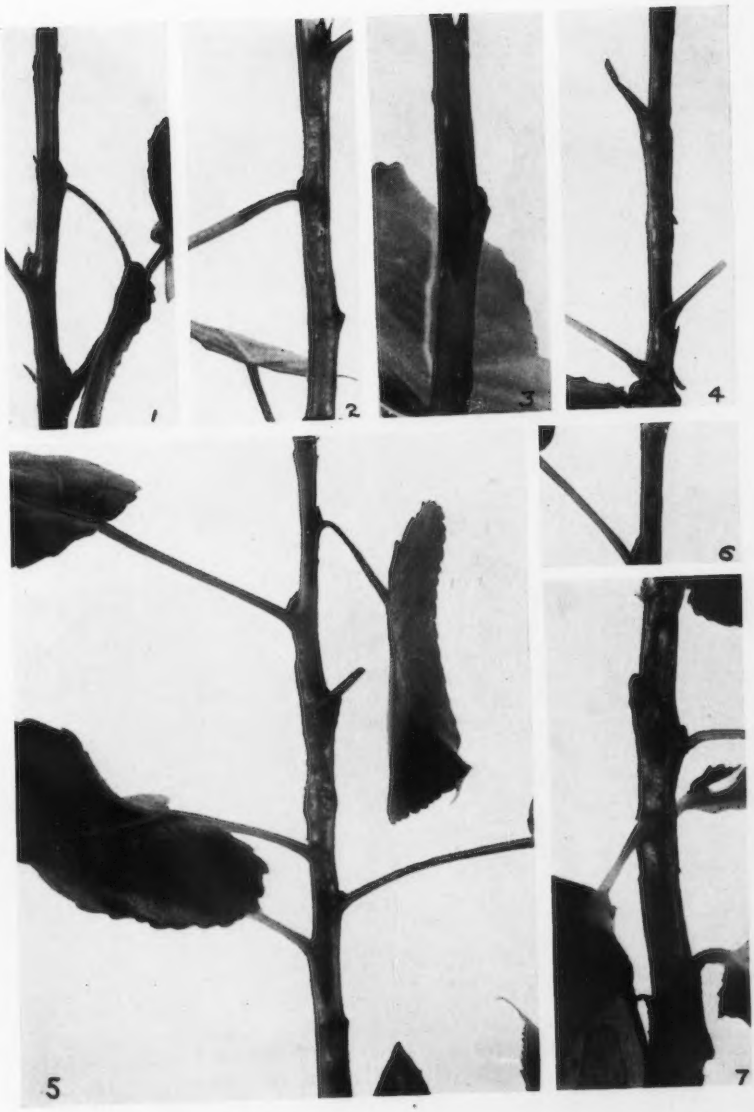
FIG. 3. *Lenticel infection on Northwest poplar.* ×¾. *Two weeks after spores were painted on the stem, slight swellings were evident around some of the lenticels. Almost immediately elongate, oval, black areas of diseased bark were produced.*

FIG. 4. *Multiple infection resulting from an inoculation on an unwounded stem of Northwest poplar.* ×¾. *On June 6, 1938, spores were painted on the leaf petioles, stipules and stem. The photograph was taken on June 26, and at this time stipule, lenticel, and petiole infection was apparent. Note the infected lenticel near the base of the cutting, and the local swelling of the entire stem arising from the infection of a number of lenticels.*

FIG. 5. *Stem wound inoculation on cottonwood.* ×¾. *A small lesion was produced as a result of the inoculation. The diseased area was soon delimited by secondary periderm tissue of the host, and no further canker growth occurred.*

FIG. 6. *Wound inoculation on P. Rasumowskyana.* ×¾. *Canker present 15 days after inoculation.*

FIG. 7. *Wound inoculation on Northwest poplar.* ×¾. *Canker present 20 days after inoculation. Note the pycnidia in the paler areas of diseased bark.*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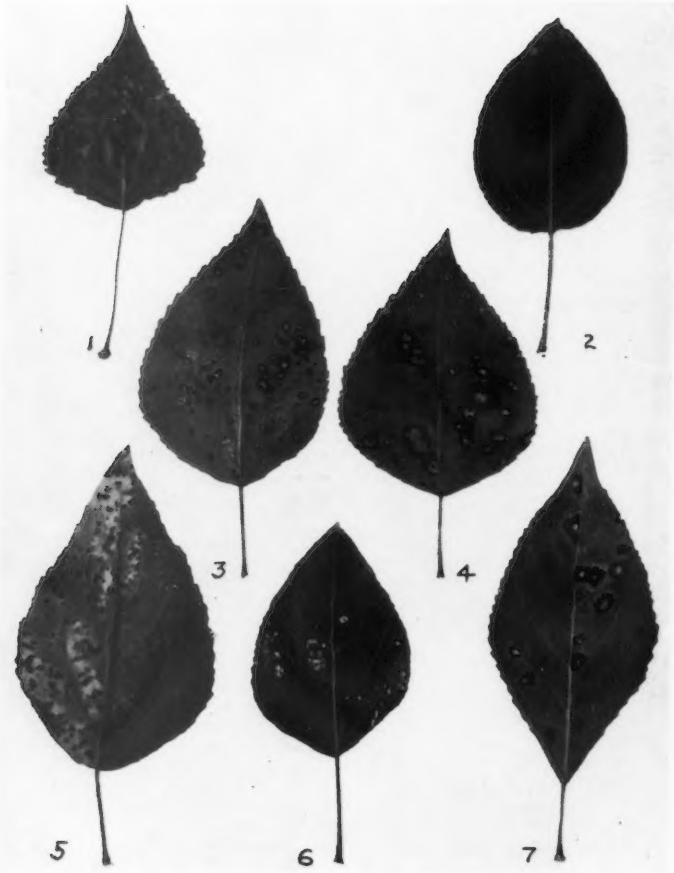

Leaf lesions resulting from artificial inoculations. $\times\frac{3}{4}$. *1. P. balsamifera. 2. P. tacamahaca 3. Northwest poplar. 4. Saskatchewan poplar. 5. P. Rasumowskyana. 6. P. Petrowskyana. 7. P. berolinensis.*

necrotic spots of various sizes which often coalesce to involve large areas of the leaf. The individual lesions may be either circular or angular, and are brown with yellowish to white centres (Plate IV). The angular spots are frequently limited by the veins. Small, black pycnidia are evident, scattered throughout the lesions on both leaf surfaces (Plate V, Fig. 2).

The Causal Fungus

Taxonomy and Morphology

The Imperfect Stage

The fungus associated with the leaf lesions and stem cankers of Russian, Northwest, and Saskatchewan poplars, agrees with Peck's (4) original description of *Septoria musiva*,* and the type specimen collected on *P. monilifera* (cottonwood), at Albany, N.Y.

The pycnidia are embedded in the tissues, with the ostioles projecting through the leaf epidermis or bark periderm (Plate V, Figs. 1, 2, and 3). In longitudinal section the pycnidial wall is evident, with the conidia borne along the base and sides of the pycnidium (Plate V, Fig. 4). The pycnidia vary in width from 64 to 120 μ, and in height from 68 to 129 μ, with an average respectively of 88 by 96 μ.

When leaves or stems bearing pycnidia are placed in a moist chamber, the spores are discharged from the fruiting bodies in the form of long, curled, pinkish cirri. The conidia are hyaline, continuous to four- (mostly two-) septate, measuring from 17.2 to 57μ long. No marked variation was encountered in the width of the conidia, which varied from 3 to 4 μ (Plate V, Fig. 5). In Table I more detailed conidial measurements are given for the fungus on cottonwood and the other hosts susceptible to canker.

TABLE I

Conidial measurements (in μ)

Host	From pycnidia on cankers			From pycnidia on leaves		
	Longest	Shortest	Average	Longest	Shortest	Average
Russian poplar	55.6	21.5	37.9 (100)	51.6	17.2	31.6 (160)
Northwest poplar	54.7	25.8	41.7 (40)	47.3	25.8	34.9 (40)
Saskatchewan poplar	51.6	30.1	38.7 (40)	43.0	25.8	36.9 (40)
Cottonwood	–	–	–	49.5	21.5	36.1 (90)

The figures in brackets after each average designate the total number of measurements taken.

In late August, September, and October, smaller embedded pycnidia are formed on the lesions. Apparently these are spermogonial structures. The fruiting bodies are filled with rod-shaped, one-celled, hyaline spores, measuring from 4 to 7 by 1 to 2 μ.

* *Dr. G. E. Thompson confirmed the author's identification of S. musiva on Russian poplar and cottonwood.*

The Perfect Stage

In September 1937, *Septoria*-infected leaves of Russian poplar and cottonwood were placed in wire baskets and overwintered out-of-doors. Some leaves were brought into the laboratory early in May 1938, and 24 hr. after they had been placed in a moist chamber, abundant ascospore discharge was obtained from small, globose perithecia embedded in the leaf tissues. The ascospores germinated and formed mycelial colonies that later produced the *Septoria* stage. Subsequent inoculation experiments confirmed the connection between the perfect and imperfect stages of the fungus. The asci, which contain eight spores, are cylindrical, short stipitate, from 51 to 73 μ long by 12 to 17 μ wide. The ascospores are hyaline, one-septate, measuring from 17 to 24 μ long by 4 to 6 μ wide. The description of the perfect stage agrees essentially with a new species of *Mycosphaerella* described by Thompson (8) as the perfect stage of *S. musiva*.

Hosts and Geographical Distribution

An important feature of *S. musiva* is that it is considered to be indigenous to North America and is not known to occur elsewhere. The preceding section has demonstrated that the pathogen is a leaf parasite on cottonwood in addition to the canker-susceptible hosts. During this investigation the fungus has also been found producing leaf injury on one or more of the following trees: balsam poplar (*P. tacamahaca* Mill.), trembling aspen (*P. tremuloides* Michx.), Balm of Gilead (*P. candicans* Ait.), and western balsam poplar (*P. trichocarpa* Torr. and Gray) in the provinces of Quebec, Ontario, Manitoba, Saskatchewan, and Alberta.

It is apparent, then, that the fungus producing the cankers on Russian, Northwest, and Saskatchewan poplars occurs as a leaf-spotting parasite on several native poplars distributed throughout the Dominion of Canada.

Cultural Studies

When suspensions of conidia or ascospores in water are poured on the surface of potato dextrose agar, the spores germinate at once, and the colonies appear within 48 hr. at room temperature. The colonies are white at first,

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Plate V

Fig. 1. *Septoria stage on a current-year twig infection.* ×8. *Note the conidia discharged in the form of cirri.*

Fig. 2. *Leaf lesion on P. balsamifera.* ×5. *Note the angular form of the necrotic area and the pycnidia scattered throughout. In some instances it is possible to see cirri extending out from the pycnidia.*

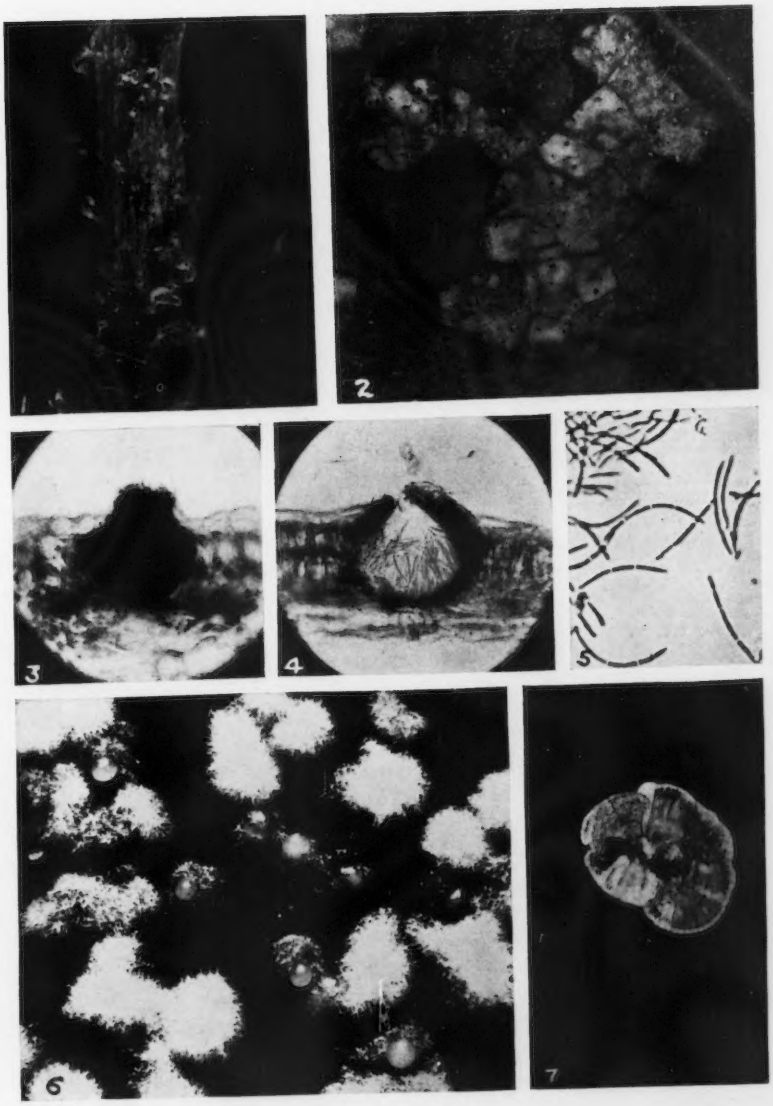
Fig. 3. *Longitudinal section through a leaf demonstrating the embedded pycnidium breaking through the leaf epidermis.* ×180.

Fig. 4. *Longitudinal section through a pycnidium showing the pycnidial wall and spores borne along the base and sides of the pycnidium.* ×220.

Fig. 5. *Conidia of S. musiva.* ×440.

Fig. 6. *Conidia produced on 4-day-old colonies growing on potato dextrose agar.* ×430.

Fig. 7. *Twenty-day-old colony resulting from the germination of several conidia.* ×1. *Note that the colony is divided into several morphologically distinct sectors.*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later turning a greenish colour except for the white, advancing margins. After the fourth day, clear to brownish drops of liquid are present on some of the colonies. These soon appear pinkish, filled with conidia from the embedded pycnidia (Plate V, Fig. 6). Mycelial colonies resulting from several spores (ascospores or conidia) germinating and growing together are frequently divided into a number of morphologically distinct sectors (Plate V, Fig. 7). Some colonies do not produce the conidial stage but remain sterile throughout their existence.

Inoculations

During the spring of 1938, inoculations were made under greenhouse conditions on potted cuttings of *P. tacamahaca*, *P. balsamifera*, *P. Rasumowskyana*, *P. Petrowskyana*, *P. berolinensis*, Northwest, and Saskatchewan poplars.

The inoculum was comprised of conidial suspensions in water, the spores having been derived from single ascospore cultures. Parallel series were conducted with inoculum from cottonwood and Russian poplar.

A number of stems were inoculated by puncturing the bark tissues with a sterile needle, and immediately applying a few drops of the inoculum to the incisions (Plate III, Fig. 6). In addition to the above inoculations, spores were painted on unwounded stems and leaves by means of a camel's-hair brush. Checks were established for each inoculation experiment. Both checks and inoculations were incubated for a period of three days, by placing the cuttings under bell-jars standing in trays partly filled with water. The cuttings were then removed and placed upon the greenhouse bench.

Results

The checks of all leaf and stem inoculations remained sterile, and the incisions healed over very rapidly. The inoculum derived from cottonwood leaves produced leaf spots and cankers similar in character to those resulting from the fungus on Russian poplar. Therefore, this evidence, supporting previous morphological and cultural studies, demonstrates that the fungus occurring as a leaf parasite on the native poplar is the same as that producing cankers on Russian, Northwest, and Saskatchewan poplars.

Leaf Spot Stage

From 7 to 21 days after inoculation, leaf lesions, identical with those found in nature, developed on *P. tacamahaca*, *P. balsamifera*, *P. Rasumowskyana*, *P. Petrowskyana*, *P. berolinensis*, Northwest, and Saskatchewan poplars (Plate IV). From two to four weeks after inoculation, pycnidia became evident. Conidia were isolated from these fruiting bodies and the resulting mycelial colonies and conidia agreed with the material used for inoculation.

Canker Stage

Inoculations on wounded stems. Two weeks after inoculation, small brownish to black areas of discoloured bark were visible around the wounds (Plate III, Fig. 6). It is important to note that these lesions were produced on the indigenous species (balsam poplar and cottonwood) in the same manner as on

the Russian and native hybrid poplars. However, a decided difference was observed in the later development of the cankers. Usually within one month the lesions on balsam poplar and cottonwood were delimited by secondary periderm tissue of the hosts, and no further development of the cankers ensued (Plate III, Fig. 4). The lesions on the Russian and native hybrid poplars continued to grow, and frequently girdled the cuttings as early as two months after inoculation. The induced cankers with their black, advancing margins enclosing paler areas with pycnidia were similar in every way to those found in nature (Plate III, Fig. 7). A general summary of the wound inoculations is presented in Table II.

TABLE II

SUMMARY OF STEM WOUND INOCULATIONS MADE DURING MAY AND JUNE, 1938

Host	No. of cuttings inoculated	Canker condition on October 10, 1938			
		No. girdled	Per cent girdled	No. living but with actively growing cankers present	No. with lesions apparently completely healed over
P. Rasumowskyana	21	17	81	4	0
Northwest poplar	15	12	80	3	0
P. Petrowskyana	13	6	46	7	0
P. berolinensis	6	1	17	3	2
Saskatchewan poplar	16	4	25	8	4
P. tacamahaca	16	0	0	2	14
P. balsamifera	24	0	0	0	24

From Table II it is evident that 80% of the cuttings of *P. Rasumowskyana* and Northwest poplar were girdled five months after inoculation. A significant decrease is apparent in the percentage girdled of *P. Petrowskyana*, *P. berolinensis*, and Saskatchewan poplar. No cuttings of the native poplars were killed, although two lesions on balsam poplar appeared active on October 10. The difference in the percentage girdled of the various hosts, supported by the observation that initially small cankers are formed on balsam poplar and cottonwood, suggest that it is not a question of absolute immunity or susceptibility of the hosts to the canker stage. It is, apparently, rather a problem of relative resistance, ranging from *P. Rasumowskyana* and Northwest poplar which may be classed as very susceptible, to *P. Petrowskyana*, *P. berolinensis*, and Saskatchewan poplar which may be considered moderatly susceptible, and finally to *P. balsamifera* and *P. tacamahaca* which appear very resistant.

Inoculations on unwounded stems. From 15 to 20 days after inoculation, lesions developed on unwounded stems and leaf petioles of *P. Rasumowskyana*, *P. Petrowskyana*, *P. berolinensis*, Northwest, and Saskatchewan poplars. This type of infection was not observed on *P. tacamahaca* or *P. balsamifera*. The stem cankers were produced at the bases of leaves and surrounding lenticels.

The first symptom of lenticel infection consisted of a swelling of the bark tissues around the lenticels (Plate III, Fig. 5). Almost immediately, elongate, oval, black areas of diseased bark were produced, (Plate III, Fig. 3), which rapidly increased in size and ultimately girdled the stems. Frequently, a number of lenticels occurring close together would become infected and produce a local swelling of the entire stem (Plate III, Fig. 5). These infections usually girdled the cuttings within two weeks following the first appearance of the symptoms of disease.

A number of cuttings were inoculated by placing conidia at approximately the mid-point along the leaf petioles. Petiole lesions resulted and the injury progressed down the petioles into the stems with the final production of stem cankers (Plate III, Fig. 1). In other instances conidia were applied to the green stipules at leaf nodes. Lesions with pycnidia were often produced on the stipules and stem cankers developed from the stipule scars (Plate III, Fig. 2).

Re-isolations were made from the infected bark and the conidia produced in cankers resulting from inoculations. The mycelial colonies and conidia obtained were of the same character as the ascospore cultures used as the inoculum.

Discussion

Broadly speaking, two distinct types of problems are encountered in the study of fungous diseases of trees. The one includes the diseases of native trees caused by native organisms, while the other has to do with the destruction of indigenous trees by introduced parasites. The results of this investigation suggest a third type which may be described as the diseases of new hosts, exotic species and hybrids, resulting from the attack of native organisms.

There is reason to believe that in the distant past indigenous trees have acquired a certain degree of resistance to native parasites, and for this reason tend to survive the diseases caused by them. The control of such diseases, therefore, will depend upon the stage of silvicultural development practised in the country.

In this study evidence is presented to demonstrate that *Septoria musiva*, a North American fungus, acts as a virulent canker-producing, as well as leaf-spotting, parasite on Russian, Northwest, and Saskatchewan poplars. The pathogen is widely distributed in Canada and occurs commonly, causing relatively unimportant leaf injury to several of the native species. This organism, therefore, is an excellent example of a native fungus, generally believed of minor importance, becoming an aggressive parasite on new hosts.

Considering the introduced trees (Russian poplars), it is apparent that the universal occurrence of the pathogen provides a continual threat to their culture, thus making control of the disease under field conditions most difficult and uncertain. This fact, in addition to widespread reports of the destruction of Russian poplars by the disease, suggests that the propagation of these species be discontinued in favour of more resistant strains.

Present evidence indicates that Northwest and Saskatchewan poplars have arisen as natural crosses between balsam poplar and cottonwood. Further, their greater ease of propagation by cuttings, more rapid growth, and hardiness point to the conclusion that they exhibit hybrid vigour. Assuming that this explanation of the origin of the hybrids is correct, it is evident that, although they possess more desirable growth characters, they are, nevertheless, susceptible to a new and serious form of disease to which the parent strains are highly resistant. It is not to be inferred that all hybrids between balsam poplar and cottonwood will be equally susceptible to the canker stage. Indeed, inoculation experiments indicated that the Northwest form was more severely attacked than Saskatchewan poplar. Therefore, if the progeny of an artificial cross between balsam poplar and cottonwood were carefully selected for hybrid vigour and disease resistance, it is quite possible that a form would be derived that possessed the growth characters of Northwest and Saskatchewan poplar, and the resistance of the parent trees.

However, it is essential that tree-breeders, and foresters favouring the importation of exotic species, take full cognizance of the possibility that each new variety may serve as a favourable host for organisms that in the past have been recognized as saprophytes or parasites of minor importance. This may result in the development of a new or more serious form of a long established disease, causing severe damage to the new host strain.

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DETERMINATION OF NITRITE, NITRATE, AND CHLORIDE IN CURED MEAT AND CURING PICKLE[1]

BY W. H. WHITE[2]

Abstract

An extract suitable for the quantitative determination of nitrite, nitrate, and chloride in cured meat was prepared by freezing and thawing the sample, followed by extraction with hot water. The sulphanilic acid-α-naphthylamine hydrochloride method, applied to the photoelectric colorimeter, was suitable for the determination of nitrite in meat extract and curing pickle. A number of factors that affect this reaction were investigated. The nitrate content of cured meat and curing pickle was determined by the phenoldisulphonic acid method, slightly modified and applied to the photoelectric colorimeter. The chloride content of meat extract was determined, either by direct titration with potassium chromate as indicator, or by Volhard's procedure, after the removal of protein by ignition or wet oxidation. The latter method should be used if accuracy within 5% is desired. Direct titration was satisfactory for the determination of chloride in curing pickle.

The precision of the above methods is illustrated by the following average deviations of individual determinations from their means, as computed from 25 or more duplicate determinations, and expressed as a percentage of the amount present, for meat and pickle respectively: chloride, ±0.20% and ±0.02%; nitrate ±0.70% and ±0.30%; nitrite, ±1.7% and ±0.1%.

Introduction

Preliminary to a survey of Wiltshire curing practice in Canada, an extensive study was made of methods for determining the nitrite, nitrate, and chloride content of cured meat and curing pickle. Many of the available procedures were found to be too laborious, or not adaptable to routine analysis, while others lacked precision or accuracy. A study was therefore undertaken with the object of developing procedures applicable to routine work, and capable of giving satisfactory reproducibility and accuracy.

Methods of the A.O.A.C. (8, pp. 354–357) for the determination of chloride, nitrate, and nitrite in cured meats require separate portions of the sample for each determination. When all three components are to be determined in the same sample, it is desirable that one extract should serve for the complete analysis. Attention was therefore given to suitable methods for preparing such an extract.

Since the concentrations of nitrite and nitrate in a cured meat extract of this kind are rather low, considerable attention was given to colorimetric methods sensitive to small quantities of these constituents. Several of the

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difficulties inherent in such procedures were overcome by the use of a photo-electric colorimeter. This paper describes a new method for extracting salts from cured meat, and advantageous modifications of existing procedures for determining the components of this extract and of curing pickle.

Preparation and Extraction of the Sample

Cured Meat

A number of possible methods for suitably preparing the sample for aqueous extraction were investigated. There is evidence from related studies that extraction is facilitated by grinding the sample with sand (11) or by freezing and thawing it (2). The effect of these two treatments was studied by passing a sample of Wiltshire bacon through a food chopper adjusted for fine grinding, until it was thoroughly ground and mixed. This material was then divided into four sub-samples, and treated as follows: (i) untreated; (ii) minced and ground with a coarse silica sand; (iii) minced, frozen, and thawed; and (iv) minced, frozen, thawed, and ground with sand. Each sub-sample was again divided for extraction with hot (boiling) or cold water. After adding 100 ml. of water to 15 gm. of each sample, the whole was shaken for 2 hr., centrifuged and the extract decanted. Nitrite and chloride determinations on each extract gave a measure of the completeness of the extraction. The average values for complete duplicate determinations are given in Table I.

TABLE I

EFFECT OF PRELIMINARY TREATMENT OF THE SAMPLE AND TEMPERATURE OF THE WATER ON THE EXTRACTION

Procedure	$NaNO_2$, %		NaCl, %	
	Cold extraction	Hot extraction	Cold extraction	Hot extraction
Minced	0.0022	0.0023	3.36	3.51
Minced and ground with sand	0.0021	0.0025	3.43	3.63
Minced, frozen, and thawed	0.0023	0.0027	3.69	3.70
Minced, frozen, thawed, and ground with sand	0.0025	0.0026	3.58	3.69

The results show that mincing, freezing, and thawing the sample give the best extraction of the salts. Although grinding with sand is better than mincing alone, no definite advantage is gained by its use in the freezing and thawing procedure. More nearly complete extraction was obtained with hot than with cold water.

The extraction proper may be accomplished by two more or less distinct methods. The first may be termed "complete" extraction, and involves extraction by shaking with successive portions of the hot solvent until the process is complete. The decanted solutions are combined, made up to volume, and an aliquot analyzed. The second method may be termed

"equilibrium" extraction, since the sample is placed in a volumetric flask with hot water, shaken for a given period, made up to volume, and a suitable aliquot taken for analysis. Such a method assumes that an equilibrium will be attained in which the quantity of salts contained in unit volume occupied by the meat will be the same as that in unit volume of aqueous extract. In this method correction for volume occupied by the dry matter may be made, but is usually negligible.

A comparison of the two procedures showed that five extractions were required for the complete removal of the salts, and that equilibrium was reached after shaking for 2 hr. Although "complete" extraction appears to have a sounder basis, the "equilibrium" method involves fewer manipulations, and is consequently more suitable for routine analysis.

A number of miscellaneous experiments on other types of extraction, and on factors that might affect extraction, were conducted. Refluxing meat samples with water gave lower results than the above procedure, although the liquid was kept definitely alkaline in order to prevent the loss of nitrite (4). Adjusting the pH of minced, frozen, and thawed samples with lactic acid or ammonium hydroxide had no beneficial effect on the "equilibrium" method of extraction.

The following procedure was therefore adopted for the extraction of salts from cured meat:

Lean meat from the sample to be analyzed was thoroughly minced, and mixed by several passages through a food chopper adjusted for fine grinding. After freezing and thawing, a 10-gm. sample was weighed into a 100-ml. beaker, a small quantity of cold water added, and the mixture worked into a paste. This was transferred to a 200-ml. wide-neck volumetric flask, and a sufficient quantity of boiling water added to bring the final volume to approximately 150 ml. The flask was stoppered, and shaken vigorously for 2 hr. in a shaking machine equipped with a steam chest to maintain the flask at a temperature of 80° C. or higher. The contents of the flask were then brought to room temperature within a period of one-half to one hour, made up to volume, shaken thoroughly, and filtered through a large fluted filter. Portions of this one extract, after suitable dilutions, served for nitrite, nitrate, and chloride determinations.

For the most part, clear extracts are obtained which do not require the use of any protein precipitant as a clarifying agent. The results of a large number of determinations have shown the method to be quite satisfactory, both in its applicability to routine work, and in its precision and accuracy. A comparison of the results given by this method with those obtained by the standard procedures of the A.O.A.C. (8, pp. 354–357) will be given later.

Curing Pickle

Since curing pickle already contains the salts to be determined in the form of a solution, no preliminary treatment of the sample is necessary.

Application of the Photoelectric Colorimeter to the Determination of Nitrite and Nitrate

Although the procedures to be described later are suitable in most cases for visual colorimetry, they have been adapted especially to the photoelectric colorimeter. The comparison of colour intensities by such an instrument eliminates many of the objections levelled at colorimetric methods, such as the necessity of preparing a number of standards, their possible variation from day to day, and the inadequacy of the human eye for accurate comparison of varying intensities of the same colour. The photoelectric colorimeter used in these investigations was that designed by Evelyn (3). If the reaction obeys the Lambert-Beer law, a constant relating the transmitted light and the concentration of the constituent may be calculated. If it does not obey this law, a calibration chart must be prepared by plotting values for concentration against corresponding galvanometer deflections. Details necessary for the use of the photoelectric colorimeter in these determinations are given below.

Spectrophotometric examination of the red colour developed in the sulphanilic acid-α-naphthylamine hydrochloride method for the determination of nitrite indicated that a Rubicon No. 520 filter, transmitting 95% of the incident light in the range 4950Å to 5500Å, was suitable. The results of a complete series of triplicate determinations on a number of standard solutions of sodium nitrite varying in concentration from approximately 1×10^{-3} to 1×10^{-4} mg. per ml. of solution gave an average value of 1.0 for K_1, and showed that the Lambert-Beer law was applicable (Table II).

TABLE II

VALUES OF CORRECTED GALVANOMETER DEFLECTION AND OF K_1 FOR THE DETERMINATION OF NITRITE

Conc. of $NaNO_2$, (mg. $\times 10^{-3}$ per ml.)	Corrected galvanometer deflection	K_1	Conc. of $NaNO_2$, (mg. $\times 10^{-3}$ per ml.)	Corrected galvanometer deflection	K_1
1.37	6.00	0.89	0.319	48.00	1.00
0.910	13.25	0.97	0.296	50.50	1.00
0.683	20.75	1.00	0.273	52.75	1.02
0.637	23.25	1.00	0.251	56.50	0.99
0.546	28.75	0.99	0.228	59.25	1.00
0.501	32.50	0.97	0.182	65.00	1.03
0.455	35.00	1.00	0.091	80.00	1.06
0.410	38.50	1.01	0.061	86.25	1.05
0.364	43.50	1.00	0.036	91.50	1.08
0·347	45.45	0.99			

A Rubicon No. 420 filter, transmitting 95% of the incident light in the region 3800Å to 4600Å, was used for the intensity measurements of the yellow colour developed in the phenoldisulphonic acid method for nitrate. Preliminary investigations indicated that the method gave low values compared to other procedures, and did not obey the Lambert-Beer law (possibly

due to occlusion of nitrate by the bulky precipitate formed). In order to overcome these difficulties somewhat, a calibration curve was prepared with standard sodium nitrate solutions, to which sodium chloride was added in order to simulate, in part, the conditions existing in meat extract and curing pickle. The results are illustrated graphically in Fig. 1 for the range from 0 to 1.00 mg. of sodium nitrate, and for a colour dilution of 100 ml.

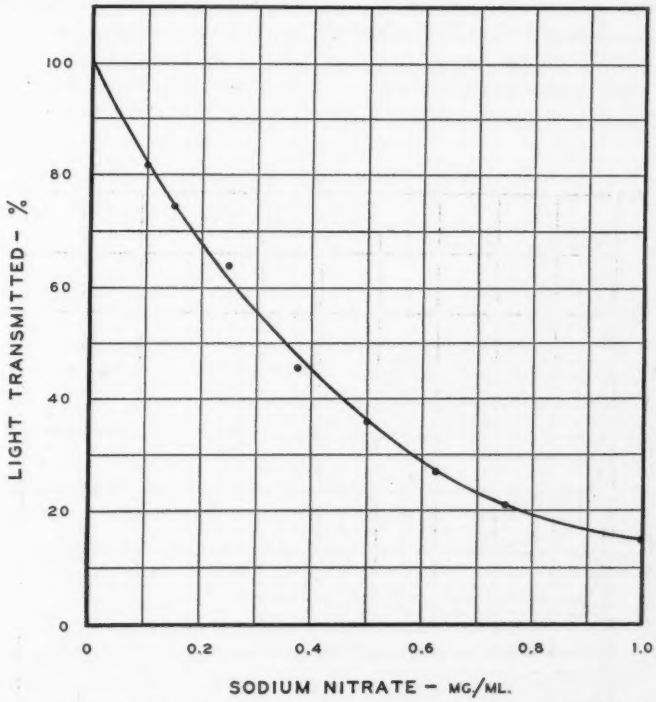

FIG. 1. *Calibration curve for the determination of nitrate by the phenoldisulphonic acid method.*

DETERMINATION OF NITRITE

Cured Meat

Of a number of colorimetric methods available for the determination of nitrite, that proposed by the A.O.A.C. (8, p. 506) was selected and found to be entirely satisfactory. To 10 ml. of meat extract, diluted to contain approximately 1.0×10^{-3} to 1.0×10^{-4} mg. of sodium nitrite per ml. of solution, was added one drop of concentrated hydrochloric acid, one ml. of a solution of sulphanilic acid, and one ml. of α-naphthylamine hydrochloride, and the tube shaken. The blank solution was prepared in the same way, but the sulphanilic acid was omitted.

The effect of a number of factors on rate of development and maximum intensity of the colour was investigated. The rate was studied by making readings of the galvanometer deflection against time for a number of solutions varying in concentration of sodium nitrite from 0.036×10^{-3} mg. to 1.37×10^{-3} mg. per ml. of solution. The results for each of the 19 concentrations indicated that the rate was very rapid during the first 5 min., and that readings made 15 min. after the addition of the reagents corresponded to maximum colour intensity. It was observed that, for the more dilute solutions, the colour had faded only very slightly even after a period of 24 hr. However, for the more concentrated solutions, the intensity had decreased considerably at the end of this period. A few of the typical curves obtained are shown in Fig. 2.

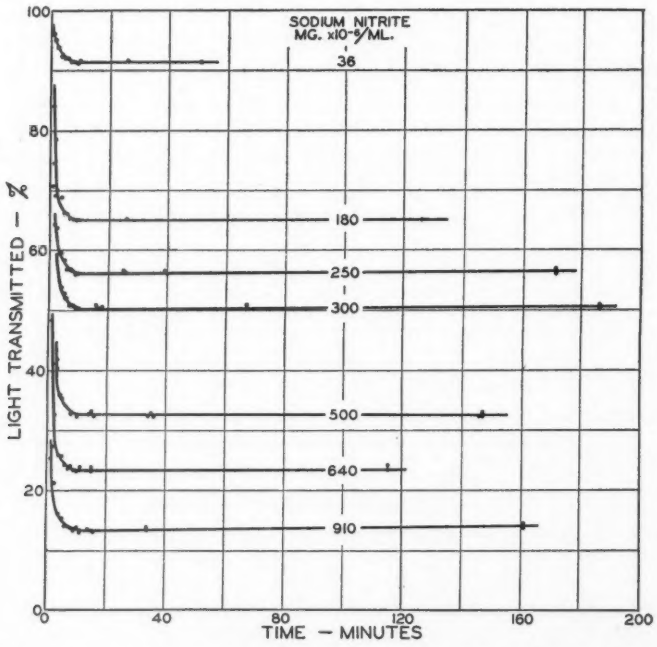

FIG. 2. *Colour development at various concentrations of nitrite in the sulphanilic acid-α-naphthylamine hydrochloride method.*

Other factors investigated were temperature, light, sodium chloride, pH, concentration of reagents, their age, and the method of their addition. The results are shown in Table III. The colour of a solution held in a steam bath developed more rapidly than that of one kept at room temperature. After reaching its maximum in 4 min., the intensity subsequently decreased, indicating that the chromogen was being either precipitated or destroyed.

Both the rate and maximum intensity of colour were increased by decreasing the intensity of incident light during colour development.

TABLE III

EFFECT OF A NUMBER OF FACTORS ON THE RATE AND FINAL INTENSITY OF THE COLOUR DEVELOPED IN THE SULPHANILIC ACID-α-NAPHTHYLAMINE HYDROCHLORIDE METHOD FOR NITRITE

Factor studied	Experimental details	Conc. of $NaNO_2$, (mg. $\times 10^{-3}$ per ml.)	
		Theoretical	Found
Temperature	Room temperature	0.185	0.185
	Heated on steam bath for 5 min.	0.185	0.181
	Heated on steam bath for 268 min.	0.185	0.095
Light	Sunlight	0.185	0.170
	Laboratory conditions	0.185	0.185
	Darkness	0.185	0.189
Sodium chloride	10% solution	0.185	0.186
	20% solution	0.185	0.187
	30% solution	0.185	0.187
pH	0.95	0.185	0.124
	1.82	0.185	0.185
	6.58	0.185	0.174
Addition of reagents	1 cc. of each, added separately	0.185	0.185
	1 cc., mixed	0.185	0.163
	2 cc., mixed	0.185	0.166
Concentration of reagents	1 cc. of each, added separately	0.185	0.185
	5 cc. of each, added separately	0.185	0.208
Age of reagents	Freshly prepared	0.185	0.185
	One week old	0.185	0.189

The presence of sodium chloride slightly increased the rate of colour development but had no effect, within experimental error, on the intensity of the fully developed colour. There is some indication that the observed increase in rate varies directly with the concentration of sodium chloride. The effect of pH was studied on three solutions adjusted to pH values of 0.95, 1.82, and 6.58, the second being the value used in all previous determinations. The rate of colour development was approximately the same for the two solutions of low pH, but was considerably retarded at the higher value. The final colour intensity was greatest for a pH of 1.82, less for 0.95, and least for 6.58.

Both the rate and final intensity of the colour are decreased by mixing the reagents prior to their addition. On a comparable volume basis, the results indicate that these two properties vary directly with the concentration of reagents employed. A solution of α-naphthylamine a week old, although considerably discoloured, gave results strictly comparable with those obtained with a freshly prepared solution.

The procedure proposed here for extraction and determination of nitrite gave results approximately 1% lower than that of the A.O.A.C. (8, pp. 356–357), but of equally satisfactory precision. The results of 25 duplicate determinations on smoked and unsmoked Wiltshire bacon, with nitrite concentrations ranging from 0.00023% to 0.013%, showed an average variation about their means of ±1.7%.

Curing Pickle

The method described above is applicable without further modification to the determination of the nitrite content of "pump", "cover", or "spent" pickle*. The use of the photoelectric colorimeter makes it unnecessary to remove any protein present which may give colour to the pickle. The results of analyses on 25 pickles of each type showed an average variation about the mean of ±0.1%, ±0.2%, and ± 0.1% for pump, cover, and spent pickle respectively.

Determination of Nitrate

Cured Meat

The nitrate content of cured meat may be determined either gasometrically or colorimetrically. Since the gasometric method requires a relatively large quantity of nitrate for accurate determination, its use would necessitate the preparation of a more concentrated extract than required for the determination of nitrite and chloride. As this was not desirable, attention was given to colorimetric procedures. Of the several possible methods, only three were thought to merit consideration. Both the brucine (10) and phenoldisulphonic acid methods (8) have been applied to cured meat. A more recently described method (7), depending on the reduction of nitrate to nitrite, and its subsequent determination, was thought to be applicable. Preliminary studies, however, indicated that the brucine and nitrate-nitrite reduction methods were both unsatisfactory because of the inconsistency of the results obtained.

Difficulty was also encountered in obtaining satisfactory results with the phenoldisulphonic acid method described by the A.O.A.C. (8, p. 356). A study of possible causes for the discrepancies observed confirmed a previous finding (1) that the solution should be alkaline during evaporation. The addition of sodium hydroxide serves a further purpose in that any excess silver sulphate present is precipitated. Nitrite interferes and is removed by oxidation to nitrate with potassium permanganate. A suitable method for the preparation of the phenoldisulphonic acid reagent is described in (9, p. 633). The details of the suggested modification of this method as applied to cured meats are given below.

To a 25-ml. portion of the extract in a 100-ml. volumetric flask, one drop of sulphuric acid (1 : 10) is added, followed by 0.6% potassium permanganate solution, drop by drop, until a pink colour remains in the extract for approximately 2 min. Chloride is precipitated with a saturated solution of silver

* *The pickle injected into the sides is designated here as pump, the freshly prepared tank pickle as cover, and that removed from the tank after cure as spent.*

sulphate added in slight excess, as indicated by precipitation on the addition of a few drops of *N* sodium hydroxide (carbonate-free). Protein material is then precipitated by adding 2 ml. of saturated basic lead acetate solution, followed by a sufficient quantity of the sodium hydroxide solution to make the solution alkaline to litmus. The flask is shaken thoroughly after the addition of each of the above reagents, the solution made up to volume, shaken, and filtered through a coarse paper until clear. (The use of a fine filter paper will result in sufficient retention of nitrate to affect the results appreciably (5).) A suitable portion of the extract, containing 0.15 to 1.0 mg. of sodium nitrate is pipetted into an evaporating dish, and taken to dryness on the steam bath.

The residue is dissolved in 2 ml. of the phenoldisulphonic acid and, after standing for 10 min., diluted with 25 ml. of cold water. The solution is made definitely alkaline with concentrated ammonium hydroxide (about 10 ml.), and transferred to a Nessler tube graduated at 50 and 100 ml. The contents are made up to either volume, depending on the intensity of the colour, shaken, filtered through a fine paper, and a portion of the filtrate transferred to a comparison tube of the photoelectric colorimeter. The blank solution, used in the initial setting of the colorimeter, is prepared by adding the same quantities of ammonium hydroxide and water to 2 ml. of the phenoldisulphonic acid reagent. As the method determines both the nitrate and nitrite present, the percentage of nitrate is obtained by subtracting that for nitrite from the total. However, the nitrite content of Wiltshire bacon is usually quite low, and may be neglected if nitrate alone is being determined.

This method for the extraction and determination of nitrates gives quite satisfactory reproducibility. The results of 25 duplicate determinations picked at random from a large number of analyses in duplicate of Wiltshire bacon (both smoked and unsmoked) showed an average variation of $\pm 0.73\%$ about the mean.

Curing Pickle

The above described method is applicable to the determination of the nitrate content of a suitably diluted portion of curing pickle. In this instance a 0.2% solution of potassium permanganate is used for the oxidation of nitrite to nitrate. The analyses in duplicate of 25 pump, cover, and spent pickles showed an average variation about the mean of $\pm 0.3\%$, $\pm 0.2\%$ and $\pm 0.4\%$ respectively.

This colorimetric method was compared with the Schlösing-Wagner gasometric procedure (8, pp. 355-356). The nitrate content of a pump, cover, and spent pickle was determined by each of the two methods. The results shown in Table IV indicate that the phenoldisulphonic acid method gives comparable results for pump, but lower values for spent and cover pickles. This is believed due to the occlusion of nitrate by the rather bulky precipitate formed by protein usually present in the last two types, but either absent, or present in a negligible quantity, in the first. The colorimetric

procedure gives closely reproducible results, but is somewhat lacking in accuracy.

TABLE IV

COMPARISON OF THE COLORIMETRIC AND GASOMETRIC METHODS FOR THE DETERMINATION OF NITRATE

Type of pickle	Analytical procedure	
	Colorimetric	Gasometric
Pump	2.73	2.73
Cover	1.82	1.93
Spent	1.08	1.15

Cured Meat

DETERMINATION OF CHLORIDE

The chloride content of a suitable portion of the extract may be conveniently determined with high reproducibility and 4 to 5% accuracy by direct titration with 0.1 *N* silver nitrate, using 1 ml. of a 5% solution of potassium chromate (8, p. 507) as indicator.

The results of 25 duplicate determinations, picked at random from a large number made on Wiltshire bacon containing from 1.8 to 6.0% sodium chloride, showed an average variation of $\pm 0.15\%$ from their means.

A measure of the accuracy of the extraction procedure, and the direct titration method for determination of chloride in the extract were obtained by comparison with the standard procedure of the A.O.A.C. (8, p. 254). Five different samples of Wiltshire bacon were each analyzed in duplicate by the following three methods:

I. The A.O.A.C. standard method: ignition of the sample followed by Volhard's determination (proteins may be eliminated by wet oxidation also (6)).

II. Twenty-five ml. of the extract treated as in I.

III. Direct titration.

The results appear in Table V, together with certain statistical quantities calculated to determine the significance of the observed differences. Although the standard error of duplicates suggests that the direct titration method is the most precise, the observed differences in these errors by the different methods are not statistically significant. However, the differences between the means by the three methods are significantly greater than their standard errors. Comparison of the results obtained with methods I and II show that the extraction phase of II is satisfactory. The higher values obtained with II may be the result of an error introduced by the impenetrable portion of the meat and its water of hydration when the extract is made up to volume. No correction has been made for this in the results presented here. On the

average the direct titration procedure gives results 4.4% too high, probably because of the presence of protein in the extract.

TABLE V

COMPARISON OF THE EXTRACTION PROCEDURE WITH THE A.O.A.C. STANDARD METHOD FOR THE DETERMINATION OF THE CHLORIDE CONTENT OF CURED MEAT

	NaCl, %		
Procedure	I	II	III
Experimental details	Direct ignition of meat; Volhard titration	Extract of meat evaporated and ignited; Volhard titration	Extract of meat directly titrated by Mohr's procedure
1	6.165	6.330	6.565
2	4.540	4.645	4.855
3	3.355	3.490	3.705
4	3.960	4.065	4.250
5	4.135	4.265	4.415
Mean	4.431	4.559	4.758
Standard error of duplicates	0.0140	0.0125	0.0100

Curing Pickle

The chloride content of any of the three types of pickle can be determined with good reproducibility and accuracy by means of the previously described direct titration procedure on a suitably diluted portion of the sample. As an independent investigation had indicated that the amount of protein commonly present in curing pickle has little effect on the Volhard method for chloride, three pickles were analyzed by both this and the direct titration procedure in order to determine the accuracy obtainable with the latter. The results, shown in Table VI, indicate close agreement between the two methods. The reproducibility is also satisfactory, since the values for 75 duplicate analyses had an average variation of $\pm 0.02\%$ about the mean.

TABLE VI

COMPARISON OF DIRECT TITRATION AND VOLHARD PROCEDURES FOR THE DETERMINATION OF SODIUM CHLORIDE IN CURING PICKLE

Pickle sample	NaCl, %		Deviation of direct titration from Volhard, %
	Direct titration	Volhard	
1	29.1	28.9	+0.69
2	31.2	31.0	+0.65
3	30.9	31.0	+0.32

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THE SYNTHESIS AND SECRETION OF PROTEIN MATERIAL BY THE PANCREAS[1]

By G. O. Langstroth[2], D. R. McRae[2] and S. A. Komarov[3]

Abstract

A mathematical treatment of the process of synthesis of protein material in the pancreas, and its secretion in response to secretin administration, has been developed. It was based on assumptions inferred from experimental data and from analogy with a suggested mechanism for the submaxillary gland, published previously. It leads to expressions that quantitatively describe the protein output in samples of pancreatic juice obtained under widely varied experimental conditions. Inferences concerning the fundamental character of certain glandular mechanisms may be drawn from the treatment. In addition it permits provisional calculation of various factors not directly observable in critical experiments, and suggests further problems in connection with the secretory processes.

Introduction

This article is mainly concerned with an attempt to interpret the data for the secretion of protein material in the experiments reported in a previous paper (4). As precautions were taken to eliminate effects due to the action of the vagus nerves or of gastric juice or bile on the small intestine in these experiments, it is considered that the data and the following interpretations refer to the behaviour of the glandular mechanism controlled by secretin.

The interpretation involves a mathematical treatment of the processes of synthesis and secretion of protein material. The premises for this treatment were suggested partly by the experimental data, partly by analogy with the behaviour of the submaxillary gland (5) and partly by general considerations. They include the following suppositions. (i) The mechanism responsible for the secretion of protein material and that controlling the secretion of water are largely independent, except as far as they both depend on the presence of secretin for their initiation. As will appear later, this view receives support from the fact that it is possible to describe the protein output in a series of samples by a theory which does not consider the secretion of water. (ii) The synthesis of protein material in the pancreas may take place at a rate comparable to that at which it is secreted in critical experiments. Synthesis is very slow in the submaxillary gland of the dog (1, 2, 3, 6); essentially complete restoration of the granules in a gland which has undergone prolonged stimulation requires from three to six days. Therefore it was permissible to neglect it in a theoretical treatment of the results obtained in critical experiments on that gland (5). When the rate of synthesis becomes comparable to the rate of secretion, however, it is an important factor in determining the

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protein output and must be taken into account. The results of experiments on the pancreas performed with a constant rate of secretin administration (4, Table I) show that the protein output per 30-min. sample decreases with time, but tends to approach a nearly constant value toward the end of the experiment. This tendency toward a constant rate of output is to be expected if synthesis is an appreciable factor, since under constant intensity of the stimulus the processes tend to reach an equilibrium state in which the rates of synthesis and secretion are equal. For reasons that will appear later, equilibrium is probably never fully attained. The strongest evidence in favour of this view of synthesis in the pancreas is that it leads to a quantitative description of the observed data in critical experiments. Synthesis of granule material must naturally occur through a chemical reaction. (iii) The secretion of protein material involves a chemical reaction or chain of reactions within the secretory cells. Attempts to explain the data on other grounds (*e.g.*, as a "washing out" of granule material) were unsuccessful. On this view, the water flow seems to remove the reaction products from the gland, and the process is similar in nature to that deduced for the submaxillary gland (5).

The mathematical treatment leads to equations that furnish a quantitative description of all the observed characteristic features of the protein output in the samples of the widely varied experiments described in the preceding paper. Inferences drawn from it allow some insight into the fundamental nature of the glandular mechanisms. In addition, it permits the calculation of various factors not directly observable in critical experiments, and is rich in suggestions for further investigations.

Formulation of the Theory

The theoretical treatment consists in obtaining velocity equations to describe the secretion and synthesis reactions, as follows:—

The secretion reaction. It is assumed that, on the influx of secretin into the cells, a chain of reactions is set up. If secretin and some other primary substance or substances take part in the first step, and if the last step results in a transformation of granule material to a form or forms readily carried out of the cells by the flow of water, then a simple form of the velocity equation describing the transformation of granule material is

$$\frac{dN}{dt} = -k^1(A_1A_2 \ldots)(B_1B_2 \ldots)N\sigma, \qquad (1a)$$

where σ and N denote respectively the amounts of secretin and granule material present in the gland at the time, and $A_1, A_2 \ldots, B_1, B_2 \ldots$ represent the amounts of other primary substances involved in the various steps of the reaction; k^1 is a constant. The equation assumes that the reaction is irreversible, and that the essential products of each step in the chain react practically as fast as they are formed. Over periods of time for which the amounts of the substances $A_1, A_2 \ldots, B_1, B_2 \ldots$, remain essentially

constant, the velocity equation reduces to

$$\frac{dN}{dt} = -kN\sigma, \tag{1}$$

where k is a constant. The term granule material, as used in this paper, refers simply to the parent substance of the secreted protein material.

The synthesis reaction. In order that the amount of granule material in the gland should not increase indefinitely it is assumed that the synthesis reaction is reversible. If a number of substances present in amounts D, E, F . . . . . combine to form granule material, present in amount N, a simple form of the velocity equation is

$$\frac{dN}{dt} = \beta\{(DEF \ldots /D_0E_0F_0)N_0 - N\}, \tag{2a}$$

where β is a constant, and N_0, D_0, E_0, F_0, ... denote the amounts of the corresponding substances present at the equilibrium point of the reaction. It may be pointed out that as the granule material may not be present in solution, possibly only the surface layers take part in the reaction; on this basis N_0 is defined as the amount of granule material capable of reacting at the equilibrium point of the reaction. A similar qualification holds in the definition of N. If the synthesis reaction in the gland is practically in the equilibrium state after a long period of rest, then for a period of subsequent stimulation such that the amounts of the substances D, E, F, . . . are essentially constant, the velocity equation (2a) reduces to

$$\frac{dN}{dt} = \beta(N_0 - N). \tag{2}$$

The above equations lead to expressions for the protein output in experimental samples without any further assumptions. As will be shown later, these expressions are in remarkably good agreement with the widely varied data of the preceding paper.

The total rate of change of granule material in the gland at any time t is given by combining Equations (1) and (2), keeping in mind the limitations imposed on these equations by the conditions of simplification from (1a) and (2a). It is

$$\frac{dN}{dt} = \beta N_0 - (\beta + k\sigma)N. \tag{3}$$

On integration, (3) becomes

$$N = N_0\frac{\beta}{\beta + k\sigma} - \frac{I}{\beta + k\sigma} \cdot e^{-(\beta + k\sigma)}, \tag{3a}$$

where I is a constant of integration. Theoretical expressions predicting the behaviour under different sets of experimental conditions are derived from (3a) and other preceding equations, as follows:

(a) Experiments consisting of an uninterrupted series of samples obtained with a constant rate of administration of secretin (e.g., (4, Table I)). For these

experiments, $N = N_0$ when $t = 0$, which determines the integration constant I of (3a). Substituting the value of I obtained from this condition in (3a), the amount of granule material N left in a gland that has been secreting under an effectively constant rate of administration of secretin for a time t from the start of the experiment, is given by

$$N = N_0\left[\frac{\beta}{\beta + k\sigma} + \frac{k\sigma}{\beta + k\sigma} \cdot e^{-(\beta+k\sigma)t}\right]. \tag{4}$$

According to Equation (1) the amount of protein material (ΔN) *secreted* during this time is

$$\Delta N = \int_o^t k\sigma N \, . \, dt.$$

Substitution of the value of N from (4), and integration, gives

$$\Delta N = N_0 \frac{k\sigma}{\beta + k\sigma}\left[\beta t + \frac{k\sigma}{\beta + k\sigma} \cdot (1 - e^{-(\beta+k\sigma)t})\right]. \tag{5}$$

The amount δN, secreted in a sample taken between times T and $(T + \Delta T)$ of this period, is of course

$$\delta N = \Delta N_{(T+\Delta T)} - \Delta N_T \, . \tag{6}$$

(b) Experiments consisting of an uninterrupted series of samples obtained under varied rates of administration of secretin (e.g., (4, Table II)). For these experiments, β and N_0 are considered to have the same value throughout, but $k\sigma$ changes on each change in the rate of administration of secretin. In the initial group of samples in which the rate of administration is kept constant, Equations (4) to (6) apply. In any subsequent group of samples obtained under constant stimulus, however, the following equations apply. For such a group the integration constant of (3a) is determined from the condition, when $t = 0$, $N = N_{i-1}$, where N_{i-1} denotes the amount of granule material present in the gland at the start of the period, and time is reckoned from this point. The amount of granule material left in the gland at a time t after the start of this period is, from (3a),

$$N = N_0 \frac{\beta}{\beta + k\sigma}(1 - e^{-(\beta+k\sigma)t}) + N_{i-1} \cdot e^{-(\beta+k\sigma)t} \, . \tag{7}$$

The amount of protein material *secreted* during a time t from the start of the period is obtained in a way similar to (5) above, as

$$\Delta N = \frac{k\sigma}{\beta + k\sigma}\left[N_0\beta t + \left(N_{i-1} - \frac{\beta}{\beta + k\sigma} N_0\right)\left(1 - e^{-(\beta+k\sigma)t}\right)\right]. \tag{8}$$

The amount secreted in a sample taken between times T and $(T + \Delta T)$ of the period is given by an equation of the same form as (6). It may be pointed out that N_{i-1} is not a new variable since it may be expressed in terms of

N_0, β, and the $k\sigma$'s; the expression is too complicated for practical use, however, and it is easier to calculate the amount of protein material left in the gland at the end of the first period from (4), and to use this as N_{i-1} for the second period. Similarly the value of N_{i-1} for the third period may be calculated from (7) applied to the second group of samples, and so on.

(c) Experiments in which the administration of secretin is stopped at some point, and the gland permitted to rest for an appreciable period before resuming stimulation (e.g., (4, Table III)). The progress of restoration of granule material during a rest period is described by the equation resulting from the integration of (2). The constant of integration is determined from the condition, when $t = 0$, $N = N_r$, where N_r denotes the amount of granule material in the gland at the start of the rest period, and time is reckoned from this point. Accordingly, the amount of granule material N_θ present in the gland at a time θ after stopping the secretin administration, is

$$N_\theta = N_0 - (N_0 - N_r) \cdot e^{-\beta\theta} . \tag{9}$$

The protein output in the samples obtained on resumption of the administration of secretin is given by Equation (8) with N_{i-1} replaced by N_θ. The value of N_r is of course calculable from Equation (4) or (7), depending on the conditions of stimulation before the rest period.

Additional formulae. While Equations (4) to (9) are those mainly concerned in a comparison of theory and experiment, the following expressions have some interest.

(a) After prolonged secretion under a constant rate of administration of secretin, the amount of protein material secreted during a time ΔT approaches a constant value δN_c, which is given by

$$\delta N_c = N_0 \frac{\beta k\sigma}{\beta + k\sigma} \cdot \Delta T . \tag{10}$$

The amount of material synthesized during the same time is given by the same expression. It is to be noted that δN_c depends on the rate of administration of secretin.

(b) The amount of granule material ΔN_s, synthesized in a time t from the start of an experiment in which the rate of administration of secretin is kept constant, is

$$\Delta N_s = N_0 \frac{\beta k\sigma}{\beta + k\sigma} \left[t - \frac{1}{\beta + k\sigma} \cdot (1 - e^{-(\beta + k\sigma)t}) \right] . \tag{11}$$

The rate of synthesis at any time is given by Equation (2).

(c) The amount of granule material synthesized in a time t after changing the rate of administration of secretin is

$$\Delta N_s = \frac{\beta}{\beta + k\sigma} \left[N_0 k\sigma t - \left(N_{i-1} - \frac{\beta}{\beta + k\sigma} N_0 \right) \left(1 - e^{-(\beta + k\sigma)t} \right) \right] . \tag{12}$$

Remarks on the comparison of theory and experiment. In experiments of type (a) the protein output in the various samples is described in terms of the unknown constants N_o, β, and $k\sigma$. The values for the constants may be determined from the observed output for three samples of a series, and these values may then be used to calculate the output in all the samples of the series. It may be added that the *relative* outputs in the samples of a series depend on only two unknowns, β and $k\sigma$, and so are fixed by the observed output in any two samples of a series.

In experiments of type (b), $k\sigma$ has a different value for each rate of administration of secretin. If the initial period of constant stimulus be sufficiently long, values for N_o and β, and for $k\sigma$ for this period may be determined as for experiments of type (a). The amount of granule material in the gland at the end of the first period may then be calculated from Equation (4) and used as N_{i-1} in Equation (8) for the second period. One then finds a value of $k\sigma$ that satisfies the data of the second period. The value of N_{i-1} for the third period may then be found from Equation (7), and the procedure repeated for the third and successive periods. This procedure—rather than a straightforward assumption of proportionality between $k\sigma$ and the rate of secretin administration—was adopted for two reasons: firstly, because the simple form of Equation (1) cannot be expected to hold indefinitely as a good approximation of (1a), and secondly, because it does not assume a strict proportionality between the amount of secretin injected and the amount present in the gland. As will be shown later, the $k\sigma$ values so obtained are closely proportional to the rate of administration of secretin, except toward the end of a long experiment, where a gradual departure occurs in the direction expected if Equation (1) fails to approximate Equation (1a) at this time.

Some Characteristic Features of the Predicted Behaviour

In order to illustrate more clearly some of the more striking features of the behaviour of a system obeying the preceding equations, the following hypothetical experiment is considered. The calculations were made with constants determined from typical experiments, and so have a general significance. The experimental conditions and predicted behaviour are indicated in Fig. 1.

The following predicted features of the behaviour of the observable factor (*i.e.*, the protein output per sample) are to be especially noted, as they will be referred to in the following section.

(i) Under constant intensity of the stimulus the protein output per 30-min. sample decreases, and finally approaches a constant value ($t = 0$ to $t = 150$).

(ii) With reduced intensity of the stimulus the protein output per 30-min. sample is reduced ($t = 150$ to $t = 240$).

(iii) With constant reduced intensity of the stimulus the protein output per 30-min. sample gradually increases and approaches a constant value ($t = 150$ to $t = 240$).

(iv) On the resumption of the initial intensity of the stimulus the protein output is greater than in the last sample of the first group, but decreases and gradually approaches the same constant value as the first group ($t = 240$ to $t = 330$).

(v) On the resumption of the initial intensity of the stimulus after an appreciable period of rest, the protein output per sample is considerably increased, but falls rapidly on continued stimulation and tends to reach the same constant value as the first group ($t = 570$ to $t = 660$).

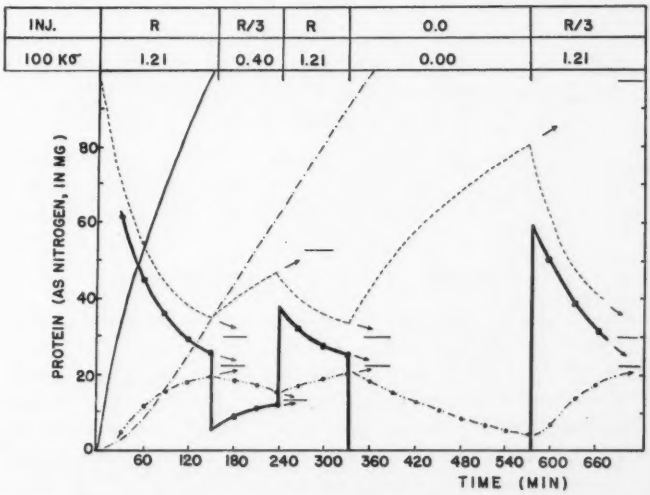

FIG. 1. *Some predicted features of a hypothetical experiment as calculated from Equations (5) to (14), with typical values for* β, N_0, *and* $k\sigma$. *As indicated at the top of the figure, five 30-min. samples are obtained with a constant rate* (R) *of injection of secretin, then three samples with a rate* $R/3$, *then three with the initial rate; the secretin administration is stopped after 330 min., and the gland allowed to rest for 4 hr., after which secretin is again injected at the original rate.* N_0 *and* β *were taken to be 98 mg., and* 0.51×10^{-2}/*min. respectively. The curves have the following significance:*

—●— *mg. of protein nitrogen secreted in each 30-min. sample* (×2).

—·●·— *mg. of protein (as nitrogen) synthesized during each 30-min. period* (×2).

– – – – – – *mg. of granule material (as nitrogen) present in the gland (see remarks concerning the definition of* N_0).

———— *total mg. of protein nitrogen secreted from the start of the experiment. Note that this becomes greater than* N_0 *in about 2½ hr.*

—·—·—· *total mg. of protein synthesized (as nitrogen).*

The arrows point to lines representing the equilibrium values which would be attained on the basis of the simplified theory if the stimulus of the immediately preceding period were maintained.

Each of these characteristic features is observed in experiments (next section) and is quantitatively described by the theory. It must be kept in mind, however, that these features are predicted by the theory which uses Equations (1) and (2) as approximations of (1a) and (2a).

Some predicted aspects of the restoration of granule material in a gland which has undergone previous stimulation are given by Fig. 2. The calculations were made using constants determined in a typical experiment, and so are believed to have a general significance.

It will be noted from Fig. 2a that for the value of β used, there is a 96% recovery within 4 hr. after the depletion of the store of granule material to 20% of its original value. In our experiments the calculated depletion has rarely been greater than 32% of the original value.

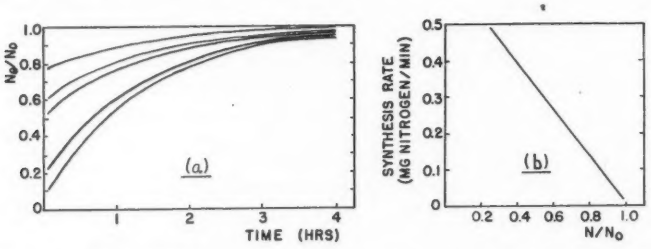

FIG. 2. *Some calculated features of the synthesis of protein material. (a) Progress of the restoration of granule material in a resting gland after various degrees of depletion (Equation 9). N_θ/N_0 denotes the amount of granule material in the gland expressed as a fraction of the amount in the "rested" gland. The time is reckoned from the start of the rest period. (b) Relation between the rate of synthesis and the amount of granule material present in the gland (Equation 2). The curves of (a) and (b) were calculated with the constants determined for Experiment 6, Table II, viz., $100\beta = 1.20$/min., and $N_0 = 54$ mg. of protein nitrogen.*

Comparison of Theory and Experiment

Tables I, II, and III compare predicted and observed protein outputs for three different types of experiment. The mode of representation is as follows. The number of each sample of secretion is given in the first column, and T denotes the time of collection (in minutes from the beginning of the experiment); "Inj." denotes the amount of secretin injected per 5 min. (in mg.); δN denotes the output of protein material in milligrams of nitrogen, in each sample.

TABLE I

AN UNINTERRUPTED SERIES OF SAMPLES OBTAINED UNDER A CONSTANT RATE OF ADMINISTRATION OF SECRETIN

Experiment 5 (see (4, Table I)): Dog, 15.4 kg.

Inj. = 6.1 $100\beta = 0.44$ $N_0 = 107$ mg. $100k\sigma = 1.06$

Sample No.	T	δN		Sample No.	T	δN	
		Calc.	Obs.			Calc.	Obs.
1	30	29.4	29.5	5	150	13.2	13.8
2	60	22.4	22.5	6	180	12.1	12.0
3	90	17.7	18.4	7	210	11.2	11.2
4	120	15.1	15.8	8	240	10.9	10.6

It may be seen from Tables I to III that the agreement between the calculated and observed values is remarkably good, although the experimental conditions varied over a wide range. In eight experiments the average deviation of the calculated from the observed outputs was only 4.5%. In

TABLE II

AN UNINTERRUPTED SERIES OF SAMPLES OBTAINED UNDER VARIED RATES OF ADMINISTRATION OF SECRETIN

Experiment 6 (see (4, Table II)): Dog, 7.5 kg.

N_0 = 54 mg.; 100 β = 1.20

Sample No.	T	Inj.	δN		100 $k\sigma$	100$k\sigma$/Inj.
			Calc.	Obs.		
1	30	6.0	14.3	14.0		
2	60	6.0	11.5	12.6		
3	90	6.0	10.3	10.0	1.00	0.167
4	120	6.0	9.4	8.9		
5	150	6.0	9.2	9.0		
6*	160	1.5	(0.88)	(1.11)	0.28	0.186
7+8	220	1.5	6.10	5.99		
9*	230	6.0	(4.2)	(2.5)		
10	260	6.0	11.4	12.4	1.10	0.183
11	290	6.0	10.4	11.8		
12*	305	1.5	(1.33)	(2.18)	0.28	0.186
13+14	365	1.5	6.18	5.81		
15*	375	6.0	(4.22)	(4.33)		
16	405	6.0	11.5	11.8		
17	435	6.0	10.4	10.6	1.10	0.183
18	465	6.0	9.7	10.1		
19	495	6.0	9.5	9.1		
20*	510	1.0	(0.72)	(1.42)	0.16	0.160
21+22	570	1.0	3.6	3.60		
23	600	4.0	6.7	7.10		
24	630	4.0	6.3	5.93	0.55	0.137
25	660	4.0	6.3	6.35		
26	690	4.0	6.2	6.35		

* *These samples were taken to clear the ducts of secretion due to the previous stimulation. Because they contain some secretion due to the previous stimulation, their observed δN values are expected to be higher than calculated on changing from a high to a lower rate of secretin administration, and lower than calculated on changing from a low to a higher rate. They are of little use in comparison of theory and experiment.*

TABLE III

UNINTERRUPTED SERIES OF SAMPLES OBTAINED BEFORE AND AFTER A 2-HOUR PERIOD OF "REST"; CONSTANT RATE OF ADMINISTRATION OF SECRETIN

Experiment 7 (see (4, Table III)): Dog, 17.5 kg.

Inj. = 8.0 100β = 0.53 N_0 = 85.3 mg. 100$k\sigma$ = 1.19

Sample No.	T	δN		Sample No.	T	δN	
		Calc.	Obs.			Calc.	Obs.
3	140*	11.1	11.2	7	380	17.3	18.4
4	170	9.8	9.9	8	410	14.2	14.5
5	200	9.7	9.7	9	440	12.2	13.7
6	230	9.1	9.4	10	470	11.1	11.4
–	230–350	Rest period†		11	500	10.4	11.0

* *This sample was taken between 110 and 140 min. from the start of the experiment.*

† *The calculated amount of granule material, as nitrogen, present in the gland immediately after collection of the 6th sample was 27 mg.; at the end of the rest period it was 54 mg. Hence the increased protein output in the samples of the latter group.*

one experiment in which the technique was not quite satisfactory, one deviation of 26% and one of 28% occurred. Aside from these two samples, the distribution of the deviations of calculated from observed values is given by

Deviation (%)	<5	5 – 10	10 – 15	>15
Number of samples	46	18	7	0

It is to be emphasized that without exception the predicted characteristic features of the protein output behaviour are observed. These features, as listed in the preceding section, are illustrated by the following experiments: point (i) by the first group of samples in Experiments 5, 6, and 7 (Tables I, II, and III); point (ii) by Experiment 6 (Table II); point (iv) by Experiment 6; and point (v) by Experiment 7 (Table III). Point (iii) is illustrated by an experiment (No. 9) that is not given in the tables. In Experiment 9, eight 30-min. samples were obtained under a constant rate of secretin administration, and then eight more were obtained with one-third the rate of administration. In the latter group the observed protein outputs, in mg. of nitrogen, were respectively 2.2, 2.6, 2.8, 2.8, 2.9, 2.8, 2.8, 3.0, while the corresponding calculated values were, 2.4, 2.6, 2.8, 2.8, 2.8, 2.9, 3.0, and 3.0. These figures clearly show the gradual rise in the protein output as predicted in point (iii). It may be added that the theory was used to design several of the experiments to test points predicted by it.

A fundamental point in the comparison of theory and experiment is that $k\sigma$ is observed to be closely proportional to the rate of administration of secretin, except toward the end of long experiments (see Table II). This finding establishes the validity of the definition of σ in Equation (1). The fact that the proportionality fails towards the end of very long experiments is to be expected, and will be discussed in the following section.

Discussion

The fact that the theoretical expressions quantitatively describe all the observed characteristic features of the protein outputs in the samples of widely varied experiments, is strong evidence that the basic assumptions of the theory are good approximations. The theory gives the following picture of the secretory processes as brought about by administration of secretin. The influx of secretin into the gland causes certain definite, and probably largely independent, processes to take place. (a) The mechanism responsible for the secretion of water is set in operation at a rate dependent in some way on the amount of secretin present in the gland. No attempt has been made by us to investigate the nature of this mechanism. In the present theory, the flow of water serves simply to carry the products of the secretion reaction into the glandular ducts, and of course to remove such crystalloids as may transfuse through the glandular membranes. (b) A chain of chemical reactions, represented by a definite velocity equation and probably irreversible, is set up in the cells. The first step involves secretin and the last results in

the transformation of granule material to a form or forms readily carried out of the cells by the flow of water. (c) As the amount of granule material in the cells is diminished by the secretion reaction of (b), a reversible chemical reaction is set up, resulting in the formation of more granule material. On this view, synthesis takes place when one of the components entering into the synthesis reaction (*i.e.*, granule material) is reduced to an amount below that existing in the equilibrium state. (d) Whether the character of the cell membranes is affected by changes in the intensity of the stimulus is uncertain, since the ease of passage of such crystalloids as sodium and potassium is so great that it might be thought to be unaffected by changes of a moderate magnitude in the membranes (*cf.*, (4)).

It is desirable to point out that the theory that describes the protein output in the submaxillary saliva of the cat (5) is only a special case of the more general theory here applied to the pancreas of the dog. The general nature of the processes is the same in both glands, although the chemical reactions involved are of course not identical. As previously pointed out, the rate of synthesis in the submaxillary gland is normally very small, but in the pancreas it is comparatively great. This may be due in part to the difference in the properties of the membranes of the two glands (*i.e.*, to the difference in rate of transfusion of substances required for synthesis).

In comparing calculated and observed protein outputs, it was assumed that the amount of secretin σ present in the gland remained constant during periods characterized by a constant rate of administration of secretin. As secretin injections were made at 5-min. intervals, however, the amount of secretin in the gland must have undergone fluctuations over a 30-min. period. In such a case the observed protein outputs may be well expressed by the equations, if σ represents the average amount of secretin in the gland during the 30-min. period. This is true only if the interval between successive secretin injections is small. It was shown to be so, by calculations based on the observed rate of diminution of water flow after stopping the administration of secretin (4, Table III). It seems reasonable to suppose that the average amount of secretin present in the gland during a 30-min. period is directly proportional to the amount injected into the femoral vein, although the proportionality constant may be expected to vary from animal to animal. This consideration is important in interpreting the $\frac{k\sigma}{\text{Inj.}}$ ratios, which are found to be essentially constant for any animal, except toward the end of long experiments.

The following remarks may be made concerning the synthesis reaction. The fact that similar results are obtained whether the parasympathetic nerves are paralysed by atropine or not, indicates that synthesis during periods of secretory activity provoked by secretin administration is not dependent on the parasympathetic nervous system. Nor is it influenced by the presence of secretin in the gland, as indicated by the constancy of β in experiments in which the rate of secretin administration was varied, and by the fact that

synthesis goes on in a regular way when no secretin is administered (Table III). According to the considerations of the section on formulation of the theory, the rate at which synthesis proceeds depends on the amounts of granule material and of certain unknown substances (the D, E, F of (2a)) present in the gland. Equation (2) with N_0 constant led to a remarkably good description of the experimental data; a comparison of (2) with the more general equation (2a) shows that N_0 of (2) stands for $(DEF \ldots / D_0E_0F_0 \ldots)N_0$. Hence the implication is that $DEF \ldots / D_0E_0F_0 \ldots$ remained essentially constant, *i.e.*, the amounts of substances required for synthesis present in the gland were not appreciably depleted during an experiment. It seems reasonable to suppose therefore that these substances were supplied continuously to the gland from an external source (*e.g.*, the blood stream), since if they originated from stores within the gland a more rapid depletion would be expected. The difference in the N_0 values found for different dogs is readily understandable, because N_0 will depend on the size of the gland and on the amounts of the substances D, E, F, . . . present in the system. It is interesting to note that N_0 is roughly determined by the weight of the dog, as might be expected (Tables I, II, and III). The β values vary somewhat from animal to animal, but why this should be so is not yet clear.

The following remarks may be made concerning the secretion reaction. If transformation of granule material to the secreted form or forms took place as the result of a direct reaction between secretin and granule material, no appreciable variation of the $k\sigma$/Inj. ratio should be expected throughout an experiment. While the ratio remains constant for a long time, it is observed to decrease gradually toward the end of long experiments (*cf.*, Table II). If the secretion reaction is a chain reaction, however, such a decrease may be expected on the following basis. According to (1a), the rate at which the secretion reaction proceeds depends on the amount of granule material, of secretin, and of certain other substances, A_1, A_2, . . . B_1, B_2, ... , present in the gland. The calculations given in the tables were made with the simplified form, Equation (1). In Equation (1), k really represents $k^1(A_1A_2 \ldots)(B_1B_2 \ldots)$, so that a decrease in the amounts of the substances A_1, A_2, B_1, B_2, present in the gland, results in a decrease in the value of k of (1), and so also of $k\sigma$. The observed decrease in the $k\sigma$/Inj. ratio is therefore attributed to a decrease in the amounts in the gland of certain substances necessary for the secretion reaction. The fact that the amounts of these substances remain essentially constant for so long a time (more than 8 hr. in Experiment 6, Table II) is considered to indicate that they are continuously supplied to the gland from an external source (*e.g.*, the blood stream).

It should be emphasized that the validity of the assumptions underlying the theory cannot be regarded as definitely established by the close agreement between calculated and observed protein outputs, nor are the interpretations given in this section necessarily unique. We have chosen the simplest assumptions and interpretations consistent with the data and with other known facts,

but it is possible that the gland behaves in a more complicated manner. If this is so, the operation of the glandular mechanisms must still be closely described by the given equations, since these fit the observed data over a wide range of experimental conditions. The chief function of the theory, and of the interpretations based on it, lies in their power to suggest new problems in connection with the secretory processes.

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